EVALUATION OF COMMERCIAL BOTTLED DRINKING WATERS IN ADDIS ABABA CITY, ETHIOPIA

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I hereby declare that this thesis entitled **"Evaluation of Commercial Bottled Drinking Waters in Addis Ababa City, Ethiopia"** was composed by myself, with the guidance of my advisor, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted, in whole or in part, for any other degree or processional qualification.

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This is to certify that the thesis prepared by **Mr. Ermias Kiros G/mariam** entitled "**Evaluation of Commercial Bottled Drinking Waters in Addis Ababa City, Ethiopia"** and submitted in fulfillment of the requirements for the Degree of Master of Science complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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I. Acknowledgements

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V. LISTS OF ACRONMS AND ABBREVIATIONS

CAC	Codex Alimentarius Commission
CES	Compulsory Ethiopian Standard
DBP’s	Disinfection by Products
EC	European Commission
EEC	European Economic Community
EPA	Environmental Protection Agency
ESA	Ethiopian Standards Agency
FAAS	Flame Atomic Absorption spectrometer
FDA	Food and Drug Administration
HACCP	Hazard Analysis for Critical Control Points
HPC	Heterotrophic Plate Count
IARC	International Agency for Research and Cancer
IDL	Instrument Detection Limit
IBWA	International Bottled Water Association
ISO	International Organization for Standardization
MDL	Method Detection Limit
MCL	Maximum Contaminant Level
MDG	Millennium Development Goals
ND	Not Detected
PCR	Polymerase Chain Reaction
PE	Polyethylene
PFA	Prevention of Food Adulteration
PVC	Polyvinylchloride

SDWA	Safe Drinking Water Act
TDS	Total Dissolved Solid
THM	Trihalomethane
WHO	World Health Organization

VI. ABSTRACT

Due to the increased demand and consumption of bottled water in Addis Ababa city, there has been a growing concern about the quality and safety of this product. The public believes and perceives that all bottled waters are safe. But it is crucial to verify whether these waters are really safe for public health or not. The purpose of the study was to investigate selected physical, chemical and microbial properties of the bottled drinking waters.

Eighteen samples representing six different brand waters were randomly collected from different supermarkets shelves for this purpose (three labeled as bottled natural mineral water and three as bottled) during the months June to July 2017.The parameters investigated were pH, Electrical conductivity (EC), Total dissolve solid (TDS), Total hardness (TH), Chloride (Cl^-), Nitrate (NO_3^-), Sulphate (SO_4^{2-}), Iron (Fe^{2+}), Calcium (Ca^{2+}) , Magnesium (Mg^{2+}) , heavy metals Cd , Cr and Fe and microbial; total coli forms (TC), Fecal streptococci (FS) using standard analytical techniques and by using standard calibrated equipments available in the selected laboratories.

It was found that the concentrations of the ionic parameters were quite different from brand to brand even in the same category; the mineral contents of most of the analyzed brands were very low, the labeled parameters were different from the actual products, there is also a violation to the standard, especially the labeling requirement .It was also observed that the standards has no lower limit for the parameters. One brand water sample was also found contaminated with coli forms. All water samples were found free of the selected heavy metals.

Key words: Bottled natural mineral water, Bottled water, Heavy metals, cations and anions, Physical parameters, TDS.

Chapter 1: Introduction

1.1 Background

Water is the most important resource to the existence of all living organisms, but this valued resource is increasingly being threatened due to several factors such as the growth of human populations and release of inappropriately treated industrial wastes, as well as demand more water of high quality for domestic purposes and economic activities.

Everyone needs water to survive, as up to 60% of the human body is composed of water. Approximately 75% of the earth's surface is covered by water, but only 1% of that is drinkable [1].This indicated that clean drinking water is not as abundant as it may seem. With water as a limited available resource and not as plentiful in some regions as it is in others, it has recently become common for water to be bottled and sold.

Water is well known to play an active role in all vital processes of the body. It allows digestion, food assimilation, waste elimination, temperature control and many others [2]. Every day we drink water or we eat watery food to replenish our metabolic reserve. Since, water is a very important element for a person's healthy life; a person must drink at an average 2.5 liters of clean and safe water daily.

According to WHO survey, 80% of all illnesses in developing countries are water-associated. Diseases caused by contaminated water consumption and poor hygiene practices are the leading causes of death among children worldwide [3]. Moreover, lack of safe drinking water, absence of basic sanitation and hygienic practices are associated with high morbidity and mortality from excreta related diseases [4]. Basically, water contaminated with pathogens, physical and chemical contaminants at the source and/or during distribution, transportation and handling in households or other working places may cause health risk when used without treatment [5].

In addition, inadequate protection of water collection and storage containers and unhygienic conditions contribute to contamination at home [6].

Currently, the quality of tap water found in Addis Ababa city is becoming deteriorated because of the inadequacy of treatment plants, direct discharge of untreated sewage into surface and ground water, and inefficient management of piped water distribution systems, and consequently, affects the physical and chemical characteristics, microbiological quality, taste and odor of the tap water [7,8]. It has the lowest water supply and sanitation coverage in Sub-Saharan countries with only 68.5% and 56% for water supply and sanitation, respectively [9]. Most of the population of Ethiopia does not have access to safe and reliable sanitation facilities. On top of these, majority of the households do not have sufficient understanding of hygienic practices regarding food, water and personal hygiene. As a result, over 75 % of the health problems in Ethiopia are communicable diseases which resulted from having unsafe and inadequate water supply, and unhygienic waste management, particularly human excreta [8].

Thus, it is timely to ask whether we can rely on drinking tap water or it is preferable to buy bottled water. Especially now in Ethiopia, there are issue associated with water pollution in certain parts and to maintain the health remedy many decide to use bottled water instead of tap water [8]. The purity, portability, safety, convenience and the mineral content is important for consumption by humans [2].

These behaviors are ideal when the bottled water is pure water that fulfills the national and international quality standards and regulations. Since water is an essential requirement of all life forms, satisfactory supply of clean, safe and hygienic water is imperative for health. Drinking unsafe and unhygienic water can cause high prevalence of waterborne diseases like diarrhea, typhoid and cholera [9]. As a preventive measure, consumption of bottled water has increased in recent years in developing countries and elsewhere. Sale of bottled water has gone to more than 35 billion US dollars and by an average of 12% increase in all over the world [10].

Prior researches have revealed that access to clean water, sanitation and hygiene are the significant elements for poverty alleviation [11, 12]. Access to safe drinking water and sanitation is a global concern. However, developing countries, like Ethiopia, have suffered from a lack of access to safe drinking water from improved sources and to adequate sanitation services [13].

Ethiopia is one of the member countries that adopted the millennium development declaration, with its main objective of poverty reduction [11]. This resulted in prioritizing accessibility to improved water supply. As a result, people are still dependent on unprotected water sources such as rivers, streams, springs and hand dug wells. Since these sources are open, they are highly susceptible to flood and birds, animals and human contamination. In addition, most sources are found near gullies where open field defecation is common and flood-washed wastes affect the quality of water in rural areas and even in urban areas the quality of water is becoming deteriorated by pollution.

According to an ADF's 2005 report, the Millennium Development Goals (MDG) objective of Ethiopia was to increase the improved water sources coverage from 2004 levels of 25% water supply and 8% sanitation to 62% for water supply and 54% sanitation by 2015[11].

As a consequence, governmental and nongovernmental organizations made efforts to construct improved sources to provide access to safe and potable drinking water. As a result of this many options to supply safe drinking water are emerging. Consumption of bottled water is common in developed countries and it starts to be introduced widely in our country in the last decades. Nowadays, though there is no definitive analysis of the reasons why people are using bottled water; three factors play important roles [14].

- Fear about the quality, safety and security of tap water
- Convenience, and availability
- Preferences and perceptions of taste etc.

In many parts of the world, tap water is either not available or safe to drink. In these regions, the failure of governments to provide basic water services has opened the door to private companies and vendors filling a critical need, even if at a very high cost to consumers. Bottled water is convenient to use, portable, reliable, and widely accessible.

In addition, sales at supermarkets, convenience stores, sporting events, and hotels put bottled water in every public location. As a result, bottled water consumption has been steadily growing in the world for the past 30 years. It is also estimated to be the most dynamic sector of all the food and beverage industry. Consumption in the world increases steadily each year in spite of its high price compared to tap water [10].

The price of bottled water is extraordinarily higher than that of tap water, but in some cases, the water quality may be similar [10]. Even, there is a chance that an expensive bottle of water is just purified municipal tap water. There happens to be skeptics of both bottle and tap, but either way, good quality drinking water is going to become harder and harder to find.

Bottled water in Ethiopia was introduced before about 15 years and during that time it was new idea and people were amazed about this product but nowadays it is becoming common and the industry is growing fast. Many feasibility studies show that the annual demand for bottled water in Ethiopia grows from less than 287 million in 2010 and forecasted to grow in to one billion liters in 2020 [14]. Similarly, the per capita consumption of bottled water by the target population in Ethiopia is expected to average 139 liters per annum. The industry's demand and supply gap reveals that supply of mineral and bottled water has been increasing at an average rate of 5% per annum. So, the need for the research on the quality of bottled water is crucial. Close examination of the industry on different approaches unveils that the bottled water industry is an emerging and fast growing sector with a huge capacity to accommodate many more manufacturers in the near future.

Existing players including the expanded capacity at their current rate of capacity utilization cannot serve more than 60% of the market demand. Indeed, this gap analysis is based on the demand for bottled water by Ethiopian consumers, without considering the demand that comes from various international and regional organizations in Addis Ababa [14]. However, recently, an increasingly worldwide concern about the quality of bottled water regarding their chemical contents has risen [15]. Especially in our country as the industry is new; the selection of the treatment system, the chemical composition of the product, the technology to analyze the parameters, the skill and awareness of the regulatory body, the consumers and the manufacturers is not well developed and these all can lead to the production of poor quality and safety products [16]. In addition, there is very limited information on the quality and safety of bottled drinking waters assessed in Addis Ababa, Ethiopia. Thus, there is urgent need to evaluate and classify the commercially available bottled waters in Addis Ababa, Ethiopia with respect to the standards to safe guard the health and safety of the consumers.

Bottled water is classified in to two groups; bottled water and bottled mineral water, the later one is further classified in to natural mineral water, carbonated mineral water and fortified mineral water [17]. Mineral content of bottled waters is one of the most important indicators for water quality and safety. Some minerals are required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health and growth [18]. Some minerals are very essential in our daily lives, which play a significant role in the nutrition of our bodies. These minerals are divided into two classes; those required in our diet in excess of 50 mg/day; designated as macro elements and those required in less than 50 mg/day called trace elements [2].

Epidemiological studies reported a strong correlation between various human diseases and the presence of trace elements in drinking water [19]. Irrespective of the above facts, in Ethiopia, the bottled waters currently found at market are not properly classified as bottled/purified or natural mineral water since the naming and classification is not as per the approved compulsory Ethiopian and other international standards [16]. In addition, there is an increasing concern about the microbial quality of bottled water marketed in Ethiopia. Most bottled waters produced in Ethiopia do not have any description related to microbial issues in their labels. Consequently, reports from sudden assessment of bottled waters collected from market indicated the detection of coli forms and heterotrophic bacteria in bottled water in counts which far exceeded from the national and international standards set for potable water for human consumption [16].

As minerals in bottled drinking waters have significant benefits to humans, de-mineralized and unsafe waters have also significant impact on health by minerals leaching effect [18]. Though many bottling companies are flourishing in the country, there are limitations in adhering and obeying to the approved standards from the bottlers' side and no proper follow up and enforcement from the regulatory body too [16]. As a result, there are doubts showing many bottled water brands containing harmful substances.

When sold in groceries or supermarkets, bottled waters all look like the same. However, there are important differences. All bottles don't contain the same product or type. There is very little in common between natural mineral water and purified water, as the chemical compositions or the treatments these waters can undergo respond to very different criteria that can change from one bottler to another.

There are many treatment systems for bottled water available like UF/ ultra-filtration, MF /micro filtration, NF /Nano filtration and RO /reverse osmosis etc. and these treatment systems must be selected to the sample water tested ahead of selection from the specific area for bottling .The problem currently happening in Ethiopia is that, most investors import the machines without exactly knowing what type of treatment system and product is required. And latter end up with a product that doesn't match the requirement then forced to put any parameters on the label which don't match the actual product , only intended good for the market. In Ethiopian context, the general objective of the study on the physicochemical and microbiological parameters of bottled water was utilized to predict the problems on the content level and framing of suitable strategies for remediation measures. Specifically, therefore, the purpose of the present study was to evaluate the physical, chemical, and microbial contents of the selected bottled waters distributed in Addis Ababa city and identifies the problems and proposes solutions on the treatment, bottling process and the standards rectified as per the national and international standards and guidelines.

1.2 Statement of the Problem

Now days, surface and ground waters are becoming polluted and contaminated by many different pollutants and contaminants as a result of urbanization, industrialization, agricultural practices, generally from point and nonpoint sources. Studies in developed countries show that cancer mortality due to exposure of ground water to hazardous chemicals is increasing; as a result a shift is occurred from municipal to bottled drinking water [20]. As the bottling business is new there is no detail studies conducted with regard to this in our country. If heavy metals , other chemicals and microbial are present in drinking water, they may lead to severe effects that include reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. Likewise in our country there may be similar issues, so it is time to investigate the bottled waters treatment system, production process, quality and safety and whether they are produced as per the standard requirement or not . It is common to see bottled waters put in direct sun light and heat at shops in our city and this by itself can be a source of health problem. Drinking water from a plastic water bottle may poses serious health risks to the consumer in relation to storage and transportation inappropriateness.

Plastic is obviously being an issue for bottled waters because of chemical leaching from the plastic to the product. When people are using bottled waters stored and transported inappropriately they may be exposed to hazardous chemicals like bisphenol A, phthalates and etc. [21]. The bottled water can also be contaminated by some heavy metals, from percolation of untreated industrial wastes, household sewerages etc. and by inappropriate treatment, production, transport and storage systems that can cause human illness and death [21]. Increased Urbanization and Industrialization are to blame for an increased level of trace metals, especially heavy metals, in our waterways.

There are over 50 elements that can be classified as heavy metals, 17 of which are considered to be both very toxic and relatively accessible. Toxicity levels depend on the type of metal, its Biological role, and the type of organisms that are exposed to it. The heavy metals linked most often to human poisoning are Lead, Mercury, Arsenic and Cadmium. Other heavy metals, including Copper, Zinc, and Chromium, are actually required by the body in small amounts, but are toxic in larger doses. The issue of safe and clean water is a big concern to governments and become a business opportunity to investors too. Though the bottling industry is mature enough in developed countries it is in an infant stage in our country with an age of about a decade and half.

As a result, the selection of the treatment technology, process used, and chemicals composition required and its analysis, equipment for testing, the standard reference used storage and transportation and facilities required etc. all have gaps to produce and deliver safe and healthy product to consumers.

There is a compulsory national standards (CES99 and CES151) adopted from the international standard for bottled water for the physical, chemical and microbial parameters as a reference. So that the regulatory body is verifying the products safety and suitability for consumption when produced and supplied as per these standards ;but currently irrespective to the standards many bottlers and consumers have lack of awareness, and knowledge on the production process, facilities and treatment systems required, testing and analysis ,labeling and naming ,storing and transporting ,chemicals composition ,and even the standard itself has limitations. These all problems may sum up to produce unsafe product that ultimately may affect the health of the consumer [22].

In addition, there is a relaxed regulation by the regulatory body, there are parameters not tested locally, like pesticides, Phthalates and THM’s etc. that makes the consumption of bottled water difficult and draws attention. As a result these chemicals are intentionally left from the scope. The study focused on problems that can be created during treatment and production process of the bottled waters as most companies may have treatment system inappropriateness, facility, hygiene and sanitation and are prone to pollution that may arise from nearby industries and human settlements.

1.3 Hypothesis /Questions

- Are the selected bottled waters sold in Addis Ababa produced as per the standard requirement and safe?
- Are the chemical compositions written on the selected bottled waters label similar to the tested parameters?
- Are the mandatory labeling and naming requirements fulfilled by the bottlers to convey reliable information to consumers?
- Is the Ethiopian bottled water and bottled natural mineral water standards complete and similar to other international standards?
- What Types of bottled water treatment methods and equipment are available and what are their advantages and disadvantages and which types are used by the selected bottlers?

1.4 Objectives

1.4.1 General Objectives

The general objective of this research was to evaluate the common physico-chemical and microbial properties of bottled waters (purified and mineral) consumed in Addis Ababa.

1.4.2 Specific Objectives

The specific objectives of the research were:

- To determine the level of cations (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+} and Zn^{2+}) , anions (Cl^{-},SO4,$^{2-}$, NO_3^{-}, PO_4^{3-}, F^{-}) , physical parameters (TDS, pH, turbidity, color and taste) and microbial detection and count of coli form, fecal streptococci and *E. coli* in the selected bottled waters.
- To evaluate the compulsory Ethiopian standard for bottled and natural mineral waters as compared to the international standards such as WHO.
- To compare the quality and safety of bottled water and bottled natural mineral waters to the national and international standards.
- To compare and determine the gap of the labeled parameters written on the plastic bottle with the study test results.
- To compare the levels of cations, anions, the physical and microbial parameters in the bottled water with the bottled natural mineral waters.
- Classify the bottled waters as bottled water or bottled natural mineral water based on their test result in comparison to the standard and to the category given by the Ethiopian conformity Assessment.
- To determine the type of water treatment systems used with its merits and demerits.

1.5 Significance of the Study

As water is essential for life, people are searching for safe and wholesome water for consumption. As a result many people perceive and are transforming their choice from municipal tap water to bottled water thinking bottled one is safe, but it may not be. So, it's increasing consumption for drinking without knowing the potential pollutants and contaminants that may be present in bottled waters initiated me to study the area. With the rapid expansion of bottled water industry in our country there may be problems and hazards in relation to the treatment process, phisco-chemical and microbial compositions, packaging, storage and transportation, standards and regulations, awareness and perception, and labeling issues etc. So The study will try to answer the problems associated selected from the above stated issues by determining the physico-chemical properties, as well as microbial detection and count together with the treatment system used both in bottled natural mineral and purified bottled waters consumed in Addis Ababa, so as to know whether the bottled waters are free from diseases causing pathogenic organisms, compounds that have adverse effects on human health and compounds that cause offensive taste and odor.

The result of the present study will serve as a baseline data for further studies on bottled waters consumption for the regulatory bodies, researchers, policy makers and consumers.

1.6 Justification

Safe drinking water is a fundamental right of human being. However, is the water that we drink safe? The answer is obviously "NO" as shown from the WHO water day report, many diseases and deaths are recorded from water borne diseases which accounts to higher percentage. Driven by the perception of purity, people switch to buy bottled water. The question is, do people get safe water as they intention from bottled water? It is doubtful. So, the current study is crucial in answering this. People have the right to know the quality of water that they perceive to be pure. Hence, this study is justifiable.

1.7 Scope of the Study

The scope of the study was encircled with the subject matter of evaluation of commercial bottled drinking waters in Addis Ababa city, Ethiopia for the selected Physico-Chemical, Microbial and Heavy metals. The geographic concentration was Addis Ababa bottled water market, particularly selected supermarkets and shops.

Chapter 2: Literature Review

2.1 Overview of Importance of Water

Water is an essential element to life on planet earth. Everyone needs water to survive, especially when up to 60% of the human body is composed of it [2]. Approximately 75% of the earth's surface is covered by water, but only 1% of that is drinkable [1]. Therefore clean drinking water is not as abundant as it may seem. Therefore, the quest for high-quality water has been an objective of human society going back to prehistoric times.

Early humans gathered in locations with readily accessible sources of water and if the water was believed to be of questionable quality, entire settlements would be abandoned. The first documented drinking water treatment can be found in Egyptian hieroglyphics (Egyptian writing system), describing procedures to purify water. The basic principles were almost the same then as they are today, boiling, chemical treatment and filtration were recommended treatments.

Although the importance of drinking water quality was known, the specific contaminants would not be identified for centuries to come. There is no such thing as naturally pure water. In nature, all water contains some impurities.

As water flows in streams, sits in lakes, and filters through layers of soil and rocks in the ground, it dissolves or absorbs the substances that it touches. Some of these substances are harmless and some are not. In fact, some people prefer mineral water precisely because minerals give it an appealing taste. However, at certain levels of minerals, just like manmade chemicals, are considered contaminants that can make water unpleasant or even unsafe. A number of chemical contaminants have been identified in drinking water.

The chemical contaminants for which epidemiologic studies have suggested a risk associated with their presence in potable water include: aluminum, arsenic, disinfection by-products, fluoride, lead, nitrate, pesticides, cadmium, mercury etc. The contaminants are of both inorganic and organic origin.

The source of the contaminant can be from point and non-point sources of pollution, naturally occurring, come from the treatment process or through materials used for packaging systems.

Naturally occurring contaminants are generally the result of leaching from geologic formations and are found primarily in groundwater. Ranges of concentrations of these contaminants range from less than Nano-grams per liter (ng/L) to milligrams per liter (mg/L).

Point sources of drinking water contaminants include direct dumping of chemicals from domestic and industrial sewage or agricultural practices. Other sources of pollution include runoff from land application of chemicals or leaching from buried solid waste landfills.

The treatment process can be a significant source of chemical contaminants. Disinfectants themselves are not believed to be a significant health hazard at levels used to treat water for drinking. The disinfectants (primarily chlorine or chlorine based), because of their strong oxidizing properties, react with the other organic constituents in the water to form chlorinated or brominated compounds believed to be of major toxicological concern. Contaminants can occur because of the distribution and packaging and storage system. As the result of leaching of distribution materials or packaging, many of the bottled waters can be found contaminated. The importance of clean water, clean air and safe working conditions initiated the public health era in the mid of 1850s. And from this concern, the science of epidemiology grew with the landmark investigation of a cholera outbreak [23]. From that filtration treatment for improving drinking water quality paralleled studies establishing the link between disease and water quality.

The introduction of chlorination of drinking water was followed by a remarkable reduction in cholera, dysentery and typhoid worldwide.

Today, water treatment and specifically chlorination and/or filtration of drinking water have been assumed as the major public health achievement of the 20th century [24].

As the century progressed, the identification of water contaminants shifted from microbiological to chemical. As the public health infrastructure grew, outbreaks associated with chemical spills or leaks increased, urbanization, industrialization drew the attention of the scientific community and finally at point treatment and bottling becomes evident [25].

Concern with inorganic contaminants such as arsenic, lead, copper and zinc began to be reported in the epidemiologic literature [26]. Nowadays in our country also diseases like cancer which were not common before are now started to be reported, these may come from pollution and contamination of food and beverages. However, for the hundreds of chemicals identified, very few have been studied or have documented proof of their health effects in humans via ingestion of contaminated water. Of the few for which a body of epidemiologic literature exists, the interpretation of the data is often confusing and controversial given the chemical of concern.

Ethiopia is one of the member countries that adopted the millennium development declaration with its main objective of poverty reduction [11]. This resulted in prioritizing accessibility to improved water supply. Prior research has revealed that access to clean water, sanitation and hygiene are the significant elements for poverty alleviation [12].

With water as a limited available resource, and not as plentiful in some regions as it is in others, it has recently become common for water to be bottled and sold. As a result, a big concern is given to supply treated water to the community and this gives opportunity to investors to be involved in bottling industry. In doing so there are problems in the treatment, processing, analysis, packaging, storage and transportation, skill and equipment to know the safety and quality of the bottled waters that needs detail researches.

There are many reasons why people buy bottled water like; fear of their tap water, taste, style, availability, convenience and security [14]. We no longer see water as a basic human right but as a product with an enlarged price sticker that we pick up in the store, rather than get from our kitchen sink, or water fountain.

Many people are becoming no longer drink from public water supplies and having easy accessibility to bottled water has decreased their demand. The more we buy bottled water, the more we are convinced that, bottled water is not a luxury, but rather a necessity [14].

We all try to avoid the things that we fear. Some people fear that, the water that comes out of their tap will harm them and fear of sickness and of invisible contamination. Due to the fear of tap water, many drink bottled water assuming it is the healthier option.

Some people have gone to drink bottled water accurately because they are concerned about their water, and the problem is they are unaware of the fact that buying bottled water is not necessarily safe, that you end up being exposed to other chemical compounds [1].

On the contrary, bottled water can actually lead to health concerns for those with a weak immune system, such as the elderly, infants, and cancer, transplant, and HIV/AIDS patients [27].

Bottled water consumption has been steadily growing in the world for the past 30 years. It is the most dynamic sector of all the food and beverage industry. Consumption in the world increases by an average of 12% each year, in spite of its high price compared to tap water [10]. Likewise some studies show, the production and consumption of bottled water in our country also increases steadily every year as can be seen in table 2.1.

Consumers may have various reasons for purchasing bottled drinking-water, such as taste, convenience, or fashion, but for many consumers, safety and potential health benefits are important considerations because they believe bottled water is safer than tap water.

There are concerns about chlorine by-products, contaminants such as lead, nitrates, and microorganism's contamination in municipal water supplies. However, some microorganisms, which are normally of little or no public health significance, may grow to higher levels in bottled waters [28].

Water is one of the most abundant and essential commodities of man, yet a greater percentage of the world's population, particularly developing countries live without access to safe water [1]. Water plays an active role in our entire vital body metabolism. It allows digestion, food elaboration and waste elimination. Although natural mineral waters have been consumed since Roman times, only the 20th century has seen the emergence of natural mineral water industry and the drinking of these products on a large scale as an alternative to tap water and non-alcoholic beverages [29].

In recent years, coping with the modern human lifestyle, there has been a tremendous increase in consumers demand for bottled mineral waters worldwide [10].

The Ethiopian conformity assessment enterprise, the Ethiopian food, medicine, and health care administration and authority are the regulatory agencies behind the bottled water supplies through the bottled and bottled natural mineral water standards.

Bottled drinking water mainly comes from groundwater, but since many municipalities use surface water as their source, and some bottlers use municipality sources, bottled water can come from surface water as well.

The ESA creates, modifies the standards for bottled natural mineral water and bottled waters that are supplied by suppliers. Until 2015, there were no proposed standards for bottled natural mineral water and sachet water. In other countries in 1996 SDWA amendments require bottled water to meet many of the same regulations as tap water for the first time [30].

However, manufacturers of bottled water, unfortunately, do not undergo the same rigorous quality standards and municipal water treatment facilities bottled water is not regulated by the EPA which is responsible for regulating public tap water supplies but by the FDA because it is considered a food product not drinking water [31].

Despite the FDA attempts to follow the EPA's tap water standards; it is not required to do so, thus allowing a greater range of bacterial contaminants to be present in bottled water [32].

If Americans are buying so much bottled water, there has to be good reasons why they are spend in substantial amounts of money on it. A lot of Americans are afraid of waterborne diseases, microbes, and dirty pathogens they do not really know anything about. The fear of tap water could come from considerable media coverage about illness due to drinking municipal tap water rather than drinking bottled, or because bottled water advertisers inadvertently suggest that tap water is inferior to bottled.

The percentage of people who drink bottled water based on the fact that they think it is safer than tap water is 35%. This is according to the American Water Works Association Research Foundation’s Consumer Attitude Survey on Water Quality Issues. Bottled water consumption in North America registered, in the last decade, an annual growth rate of 25% [33]. Bottled water is often expected to be purer compared with tap water, although this is not necessarily always the case.

Public awareness about waterborne diseases and poor quality control of drinking water has been increased [34].The presence of opportunistic pathogens such as *Pseudomonas aeruginosa* in mineral waters underscores the importance of caution regarding the safety of these products, especially for health compromised individuals.

Drinking Water

Drinking water, or potable water, is defined as having acceptable quality in terms of its physical, chemical, bacteriological parameters so that it can be safely used for drinking and cooking [35]. WHO defines drinking water to be safe if and only if no any significant health risks during its lifespan of the scheme and when it is consumed.

Table 2.1. National Annual Bottled Water Demand Forecast (2010-2020). [36]

Year	Population	Consumption in Liter Per person	Total Annual Demand in the Country (Liters)	Remark
2010	79,455,634	4.40	349,604,790	Base Year
2011	81,521,480	5.02	408,911,746	
2012	83,641,039	5.72	478,279,535	
2013	85,815,706	6.52	559,414,875	
2014	88,046,914	7.43	654,314,014	
2015	90,336,134	8.47	765,311,844	
2016	92,684,874	9.66	895,139,345	
2017	95,094,680	11.01	1,046,990,783	
2018	97,567,142	12.55	1,224,602,300	
2019	100,103,888	14.31	1,432,343,834	
2020	102,706,589	16.31	1,675,326,642	

Bottled Water

Packaged/bottled drinking water other than bottled natural mineral water is water derived from any potable source which may be subjected to treatments such as, decantation, filtration, combination of filtrations, aeration, filtration with membrane filter, cartridge filter, sand filter activated carbon filtration, demineralization, re-mineralization, reverse osmosis or any other method to meet the prescribed standard and packed. It may be disinfected to a level that will not lead to harmful contamination in the drinking water.

The potable water used for production of packaged drinking water is water derived from any source (such as ground water like bore well, public drinking water systems such as municipality supply or supplies from other sources) received on regular basis and is intended for human consumption for drinking and cooking purposes. It includes water (treated or untreated) supplied by any means for human consumption [37].

As indicated above, the packaged drinking water can also be produced by way of re-mineralization. This process involves addition of ingredients. In case re-mineralization is carried out by any manufacturer, ingredients used for the purpose shall be of food grade quality conforming to the requirements of the PFA Act, 1954 and the rules framed there under.

Processed water may be disinfected by means of chemical agents and/or physical methods to control the micro-organisms to a level that does not compromise food safety or suitability for consumption. Various means adopted for disinfection include ozone treatment, ultraviolet treatment, etc. and/or combination thereof. The processed water shall be filled in sealed containers of various types/sizes/shapes made from the plastic materials permitted under WHO and CES 99 in our country suitable for direct consumption without further treatment. The filling & packing of the processed water shall be in containers which are tamperproof, tight and impervious. The containers with features like Jugs, Jugs with built-in taps, Jars with threaded (reusable) caps, bottles etc. which are not tamperproof and leak proof shall not be permitted. There are many terminologies presently adopted by the industry & consumer for describing the processed water as packed in different packaging. For the purpose of uniformity in describing the various types of containers, the following definitions are suggested [38].

Table 2.2. Bottled water container types [38].

Type of container	Description
Jars	Reusable plastic containers
Bottles	One time use plastic containers, to be crushed after use
Cup	One time use plastic container in the shape of cup or glass/tumbler that is to be crushed after use.
Glass Bottle	Containers made of glass material which are to be used after sterilization)
Pouch /sachet	Containers made of PE plastic in the shape of a bag to be crushed after use

a. Pouch b. cap c. bottle d. jar

Figure 2.1. Different types of water containers naming [38]

2.2 Bottled Water Types:

a. **Bottled drinking water (other than packaged natural mineral water):** It is water that is intended for human consumption and that is sealed in bottles with no added ingredients except that it may optionally contain safe and suitable antimicrobial agents.

b. **Natural mineral water:** It is water clearly distinguishable from ordinary drinking water because of the following characteristics:

- It is characterized by its content of certain mineral salts and their relative proportions and the presence of trace elements or of other constituents.
- It is obtained directly from natural or drilled sources from underground water bearing strata for which all possible precautions should be taken within the protected perimeters to avoid any pollution of, or external influence on, the chemical and physical qualities of natural mineral water.
- Of the constancy of its composition and the stability of its discharge and its temperature, due account being taken of the cycles of minor natural fluctuations.
- It is collected under conditions which guarantee the original microbiological purity and chemical composition of essential components.
- It is packaged close to the point of emergence of the source with particular hygienic precautions.
- It is not subjected to any treatment other than those permitted by this standard.

c. **Naturally carbonated natural mineral water:** It is a natural mineral water which, after possible treatment and re-incorporation of gas from the same source and after packaging taking into consideration usual technical tolerance, has the same content of carbon dioxide spontaneously and visibly given off under normal conditions of temperature and pressure.

d. **Non-carbonated natural mineral water:** It is a natural mineral water which, by nature and after possible treatment and after packaging taking into consideration usual technical tolerance, does not contain free carbon dioxide in excess of the amount necessary to keep the hydrogen carbonate salts present in the water dissolved.

e. **De-carbonated natural mineral:**

It is a natural mineral water which, after possible treatment and after packaging, has less carbon dioxide content than that at emergence and does not visibly and spontaneously give off carbon dioxide under normal conditions of temperature and pressure.

f. **Natural mineral water fortified with carbon dioxide from the source:** It is a natural mineral water which, after possible treatment and after packaging, has more carbon dioxide content than that at emergence.

g. **Carbonated natural mineral water:** It is a natural mineral water which, after possible treatment and after packaging, has been made effervescent by the addition of carbon dioxide from another origin.

h. **Natural spring water:** It is derived from an underground formation from which water flows naturally to the earth's surface.

i. **Distilled water**: Distilled water is a type of purified water. It’s water that has gone through a rigorous filtration process to strip it not only of contaminants, but any natural minerals as well. This water is best for use in small appliances, like hot water urns, or steam irons, because if you use it, you won’t have that mineral buildup that you often get when you use tap water. Though it may seem counterintuitive, this water is not necessarily the best for human consumption, since all of the water’s natural, and often beneficial, minerals are absent.

j. **Purified water**: Purified water is water that comes from any source, but has been purified to remove any chemicals or contaminants. Types of purification include distillation, deionization, reverse osmosis, and carbon filtration. Like distilled water, it has its advantages and disadvantages, the advantages being that potentially harmful chemicals may be taken out and the disadvantage being that beneficial minerals may be taken out as well [38].

2.3 Bottled Water Quality Characteristics

The market is becoming busy with a large number of brands of bottled waters. Various countries have enforced drinking water standards for the maximum permissible levels of different constituents. Due to increased demand and consumption of bottled water there has been a growing concern about the quality of these products.

In recent times concerns have been expressed about the increase in poor quality of well water due to the nitrate pollution through continuous and liberal use of organic manure and inorganic fertilizers [39].

In Ethiopia there is also a problem regarding the quality of the bottled waters with respect to some heavy metals and microbial quality of these products. The ECA in 2015; announced that they closed many factories in relation to product quality and safety problems [16]. Therefore, recently consumption of bottled water has been increased. But, the quality of bottled water used for human consumption is not subjected to any stringent quality control measures. Recent study by the IBWA revealed that 25% of all bottled waters are simply tap water placed in a bottle which is a valid method of bottling water by the FDA under certain Good Manufacturing Practices regulations.

In addition, the quality of bottled water substantially vary among brands as well as with time and with different production runs depending on its source, treatment technology, manufacturing operation, packaging material and shelf-life and best before use [40].

Although, bottled water should have a shelf life of 30 days unopened, most bottled water companies' label showed that their water is valid for 1 to 2 years. On the other hand, bottled water is most commonly disinfected with ozone, which provides a residual disinfection for a limited time and subsequently does not leave a residual taste like tap water, which uses chlorine as a final disinfectant.

The length of time chlorine and ozone remains active in water depends on many factors, including temperature. However, bottled water may be in distribution and storage conditions for several weeks which may adversely affect its quality [41].

2.3.1 Microbiological Quality of Bottled Water

Water should be free from any microbes. But unfortunately water is not always found pure. The contamination of natural water with fecal material, domestic and industrial sewage as well as agricultural and pasture run off may result in an increased risk of disease transmission to humans [42].

The microbiological quality of bottled water is of great interest as many consumers use it as an alternative to tap water and consider it to be better and safer. The quality of water is determined largely by bacteriological analysis. In bottled waters, the bottling process may be a source of additional contamination. In addition, the common sources of contamination of bottled water are equipment, bottles and caps, exposure to air and contact with humans during the bottling process [43].

Although the microbial quantity levels in processed water are often initially low, they can evolve rapidly to high levels during storage [44].

This rapid growth of bacteria after the water is bottled may be due to oxygenation of the water during processing, the increase in surface area provided by the bottle, the increase in temperature and the amount of nutrients arising from the bottle [45]. There are reports that reveal, some bacteria can multiply on polyvinyl chloride of ultra-marine blue dye in bottle plastic material. Another factor to be taken into account is whether the water is carbonated, since the decrease in pH resulting from carbonation acts to prevent bacterial growth [46].

Bottled drinking water is defined as natural water packaged in bottle that has definite physical, chemical and microbiological specifications. These specifications are met through physical treatment. Bottled water must be at least as good in bacteriological quality as potable water and thus the total bacterial count should not exceed 50 cells ml^{-1} at 37°C after 24h. It should also be free from coli form organisms, *E. coli, E. faecalis, P. aeruginosa* and *C. perfringens* [47]. Several methods are used for bottled water to meet the required specification. Among these methods are filtrations by using special kinds of filters and ultraviolet exposure. Although modern treatment processes and stringent regulations mean that tap water is generally safe to drink in developed countries, microbial pathogens remain the major public health risk associated with drinking water.

Major waterborne pathogens include bacteria (*e.g*, pathogenic *Escherichia coli*, *Campylobacter* and *Salmonella* spp.), protozoa (*e.g.*, *Cryptosporidium parvum* and *Giardia lamblia*), and viruses (*e.g.*, adenoviruses, entero - viruses and Rota-viruses) [48].

In addition to that many filamentous fungi appear in various surface water and underground water, but during the last years they have been found in various drinking water as well [49].

Epidemiological studies have reported the occurrence of disease including problems with reproduction, cancer, congenital malformations of the central nervous system, cardiovascular disease and even death due to exposure to trace elements and mineral contents of water [49].

So this shows with the rapid increase in number of the bottling companies must be with due care. In general, people are concerned about the poor quality of tap water that is why they have switched over to bottled water perceiving it to be clean and safe. Although, people consider bottled water is safe but it can be also be contaminated with chemical and biological agents.

Presence of coli form bacteria, *E.coli or Pseudomonas* in bottled water can pose a great threat to the public health. Infants, young children, debilitated and immuno-compromised people are at high risk of waterborne diseases, even at lower infective doses [27].

So for this purpose the bottling companies must put in their label the intended use or target user, unfortunately we don’t get it.

Water borne diseases are one of the major health problems especially in developing nations. The high prevalence of disease such as diarrhea, typhoid fever, cholera and bacillary dysentery has been linked to the consumption of unsafe water produced under unhygienic production practices. Water quality is generally defined as also the physical, chemical and bacteriological characteristics of water in relation to the requirements to human need.

Pseudomonas aeruginosa has been found in some mineral waters in various countries such as Brazil, Canada, France, Germany, Spain, United States and others counters. Also, examination of drinking water for *Pseudomonas aeruginosa* is not recommended as a routine procedure, but it can be used as an indicator of good manufacturing processes and suitability for drinking water.

Bacteria belonging to the genus *Pseudomonas* are widely spread in the environment and are often opportunistic bacteria for many episodes of infections. During the period of storage the growth of *Pseudomonas aeruginosa* may lead to a risk for consumers especially the immunologically weak persons, as well as very young or elderly ones [41].

In addition, reported that, the initial microbial counts of the examined bottled water moderately increased during the increase of storage time at room temperature. Improvement of the quality of bottled water will be achieved only by improving the manufacturing processes and subsequent storage condition [50].

A survey of bottled water conducted in the United Arab Emirates, where about 90% of the populations drink bottled mineral water; showed that out of 2.0-1.5 liters bottles, 40% were contaminated by bacteria. However, new researches claimed that; not all bottled waters are pure, for example it is found that, of the thirty bottled samples tested in Saudi Arabia, 2 of 9 bottled samples showed contamination with *Bacillus cereus* and *Pseudomonas* [51].Therefore, this shows there is great threat with the expansion of bottling companies in our country needs serious attention in research and regulations.

The most common and widespread health risk associated with drinking water is contamination, either directly or indirectly, by human or animal excreta, and with the micro-organisms contained in the excreta. Monitoring of specific bacterial, viral and protozoan pathogens is usually complex, expensive, and time consuming, and may fail to detect their presence. In monitoring for microbiological quality, reliance is therefore placed on relatively rapid and simple tests for the presence of indicator organisms. The three common organisms used as microbial indicators are total coli forms (TC), thermo tolerant coli forms (TTC) or alternatively *E. coli* and Enterococcus [52].

Total Coli-form: - Total coli form (TC) bacteria comprise many members of the family Entero-bacteraceae. TC bacteria are those that can grow in selective media at 35°C and ferment lactose or possess a B-galactosidase enzyme, as an indicator of fecal contamination. They are not useful as an index of fecal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms [53]. On the one hand, the Total Coli form group of bacteria is unreliable indicators of fecal contamination because many members are capable of growth and long term persistence (having a non-fecal origin) in many environments, including water distribution systems. On the other hand, there are more TC bacteria in untreated fecal waste than any of the other fecal indicators or indicator groups, making the TC test the most sensitive of all indicator tests. Because of this sensitivity, the TCR (total coli form rule) relies on the TC bacteria test as the initial test to detect the possible presence of fecal contamination in delivered water, as well as to assess water treatment effectiveness and the integrity of the distribution system.

Water from a distribution system that is free of TC bacteria should have no or minimal levels of pathogens.

Fecal Coliform: - Under the TCR, if the TC test result is positive, that sample is then further tested for the presence of fecal coliform (FC) bacteria. Since it is difficult to monitor disease carrying microorganisms directly we use the count of FC bacteria as a standard measure and indicator of disease potential. The presence of FC bacteria in water indicates that fecal material from mammals or birds is present, so organisms that cause water born diseases may be present as well. The FC group of organisms is a subset of the TC group that can grow in selective media at 44.5°C and ferment lactose, majority of FC bacteria are E. coli [54].

Enterococcus: Enterococci are facultative organisms, *i.e.*, they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments. Though they are not capable of forming spores, enterococci are tolerant of a wide range of environmental conditions; extreme temperature (10-45°C), pH (4.5-10.0) and high sodium chloride concentrations [55].

Table 2.3. Water borne pathogens and their associated diseases [56].

Pathogen		Diseases
Bacteria	Campylobacter spp.	Diarrhea and acute gastroenteritis
	Enteropathogenic Escherichia coli	Diarrhea
	Escherichia coli O157:H7	Bloody diarrhea and hemolytic uremic syndrome
	Salmonella spp.	Typhoid fever, diarrhea
	Shigella spp.	Dysentery, diarrhea
	Vibrio cholera	Cholera, diarrhea
	Yersinia spp.	Diarrhea, gastrointestinal infections
Viruses	Adenoviruses	Diarrhea, respiratory disease, conjunctivitis
	Astroviruses	Diarrhea
	Coxsackie viruses (Enterovirus)	Respiratory, meningitis, diabetes, diarrhea, vomiting, skin rashes
	Echoviruses (Enterovirus)	Meningitis, diarrhea, myocarditis
	Enteroviruses 68-71	Meningitis, diarrhea, respiratory diseases, rash, acute enteroviral haemorrhagic conjunctivitis
	Hepatitis viruses (A, E)	Hepatitis (jaundice), gastroenteritis
	Caliciviruses	Diarrhea, vomiting
	Poliovirus (Enterovirus)	Poliomyelitis
	Rotaviruses	Diarrhea, vomiting
	Small Round Structured viruses	Diarrhea, vomiting
Protozoan parasites	Cryptosporidium parvum	Cryptosporidiosis, diarrhea
	Entamoeba hystolytica	Amoebic dysentery
	Giardia	Giardiasis, diarrhea
Helminths	Dracunalis medinensis	Guinea worm (Dracunculiasis)
Emerging opportunistic pathogens	Actinobacter spp.	Septicemia, meningitis, endocarditis
	Aeromonas spp.	Diarrhea, gastroenteritis
	Cyclospora spp.	Diarrhea, abdominal cramping, fever
	Isospora spp.	Diarrhea
	Legionella spp.	Legionnaires disease, Pontiac fever
	Microsporidia spp.	Gastrointestinal infections, diarrhea
	Nontuberculosis Mycobacteria	Skin infections, cervical lymphadenitis, nontuberculosis mycobacterium disease
	Pseudomonas aeruginosa	Septicaemia, wound and eye infections

2.3.2 Physico-Chemical Water Quality Parameters

The water formula H_2O, two atoms of hydrogen and one atom of oxygen is only found in distilled water with this structure. Rain water, snow and ice are quite similar to distilled water. Water presents in nature contains, even if in traces, minerals very important for our health, salt and oligo-elements dissolved during its way through the soil or it's flowing in rocky streams [57].Our body is constructed of these minerals which also play crucial role in our body activities, figure 2.2 shows the mineral composition of a 60 Kg body.

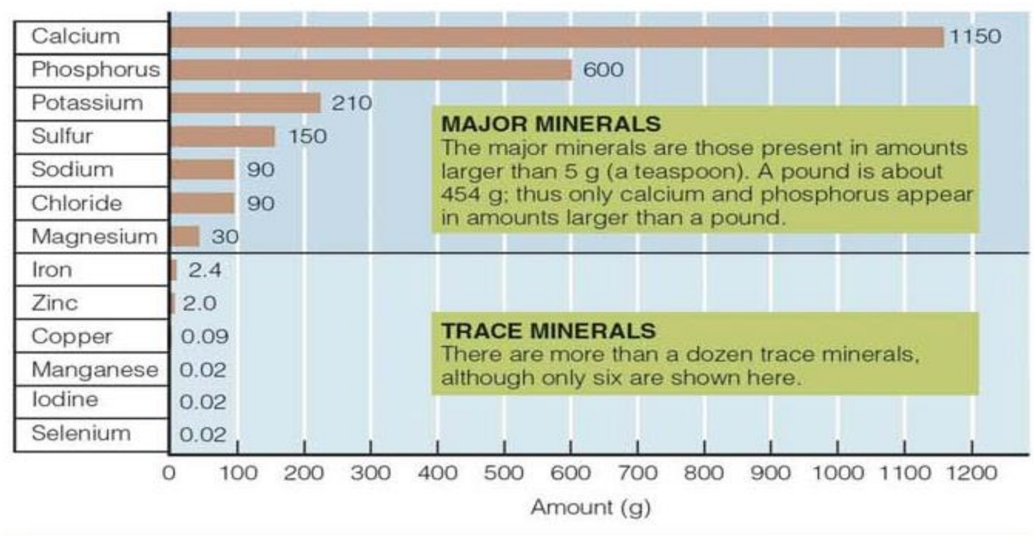

Figure 2.2. Minerals in a 60Kg human body [2]

Calcium: Calcium is one of the most common elements on the earth [58]. It is essential in our body for teeth and bones formation, blood coagulation, right functioning of our nervous system. Calcium ions are contained in almost all spring drinking water. Health effects caused by hard water, very rich in calcium and magnesium, are unknown [59].

An excess in calcium can alter the water taste or cause scaling problems in pipes and household appliances.

In the reduction of the content of calcium and magnesium ions dissolved in water it is recommended that the calcium content never goes under 60 mg/L. The World Health Organization has recommended a minimum calcium daily intake of about 700 mg. Drinking calcium poor water is considered dangerous for the risk of coronary diseases.

Magnesium: Magnesium, with sodium and calcium, among the cations most commonly found in drinking water. In humans magnesium is important for many metabolic functions and for muscular and nervous activity [59]. The daily recommended intake is 150-500 mg.

Sodium: Sodium is an element very diffused on earth and in the biosphere; even if in nature it is almost never in its pure form, but mainly in form of salt (NaCl). Our body contains an average of 100g of sodium which is an important metabolic regulator for nervous and muscular stimulations. The daily sodium chloride intake is 200 mg. Due to our diet very rich in salt it is recommended to drink water with sodium content lower than 20mg/L, particularly for hypertension people and children. The salt consumption in industrialized countries is considered much higher than the recommended levels (about 3.9 g/day on average) [59].

Drinking 2 liters of water containing 20mg/L of sodium you reach 40 mg that is about the 5% of the total intake. To reduce the daily sodium intake it would be more logical to change your nutrition, i.e. to eat only integral sea salt, more equilibrate and rich in mineral salts at home, and to avoid precooked food, always rich in refined salt.

Chromium: Chromium is an important oligo-element for our organ, on condition that certain concentration is not exceeded and the element is not found in toxic or carcinogenic combinations (always due to industrial pollution). Chromium speciation in environmental samples is of interest, because its toxicity to aquatic and terrestrial organism depends on the oxidation number of chromium. Chromium is an industrially important metal, which has the potential to contaminate drinking water sources. Chromium (VI) is more water soluble, more easily enters living cells, and is much more toxic than chromium (III). Chromium (VI) is a human carcinogen, as determined by the National Toxicology Program, the International Agency for Research on Cancer, the U.S. Environmental Protection Agency. Chromium enters environmental waters from anthropogenic sources such as electroplating factories, leather and textile manufacturing facilities. Chromium also enters groundwater by leaching from soil [60].

Iron: Food rich in iron is very important, particularly for children and women in fertile age. The recommended daily intake is 10 mg. Iron is usually contained in low amount in drinking water. The WHO has recommend a maximum of 2mg/L the EEC of 0.2 mg/L. Possible increasing are not to be considered harmful, even if they make the water not nice to drink and give an unpleasant reddish color.

Chlorine: At present chlorination is the most used treatment to remove water bacteria which could cause health problems. The Italian law allows 30 mg/L of chlorine, while the guidelines of the European Directive indicate 1 mg/L and specify that the concentration should be as low as possible. According to international research; the consumption of water containing compounds formed after the reaction between chlorine and microorganisms, can contribute to the increasing of urogenital tumors. When the tap is opened the smell of chlorine odor typical of swimming pools is recognized, it is recommended to pour the water in a large tank and to leave it open or semi-open for about half an hour to mix.

Fluorine: In someone opinion fluorine is useful for the good health of bones and teeth, sometimes it is even essential, in others opinion it is unnecessary when you are adult, above all if it is added. Fluorine is a halogen and it is the most electronegative of all the elements, so it reacts easily with most of the elements. In 1945 the addition of fluorine in drinking water began to be experimented in New York State, followed by Australia and some areas in UK, with the declared purpose of preventing dental caries in population [61]. Water fluorination is prohibited in Belgium, Denmark, the Netherlands and France; in Spain and Germany local authorities handle every decision, and in Italy there is no specific law on this matter. Fluorine values useful for our body are very close to toxic values, so a dispense not aimed and personalized can cause high risk of overdosing and chronicle poisoning, with consequent skeleton deformation, spots on tooth enamel, neurological disorders, damages on the thyroids and even tumors. Fluorine has negative effects on the central nervous system, determining behavior alterations, cognitive deficit, influencing on the fetus development even in concentration not harmful for the mother.

Zinc: Zinc is one of the most common elements in the earth's crust. It is also an essential element for all living things [62]. Pure zinc is a bluish-white, shiny metal. Zinc has many commercial and industrial uses. A large proportion of all zinc, perhaps more than a third, is used to galvanize metals such as iron so as to prevent corrosion.

Zinc metal is used for dry batteries, roof cladding, and to protect iron structures from corrosion by attaching zinc as sacrificial anodes [62]. Zinc is mixed with other metals to form alloys such as brass and bronze, and pennies are made from a copper-zinc alloy.

The oxide (ZnO) is also combined with other elements such as chlorine, oxygen, and sulfur to form zinc compounds used to make white paints, ceramics, rubber, wood preservatives, dyes, and fertilizers. Zinc compounds are also used in the drug industry as ingredients in common products like sun blocks, diaper rash ointments, deodorants, athlete's foot preparations, and anti-dandruff shampoos. The sulfide (ZnS) is used in making luminous dials, X-ray and TV screens, paints and fluorescent lights. According to EPA, in natural surface waters, the concentration of zinc is usually below 10μg/L, and in ground waters, 10–40μg/L. In tap water, the zinc concentration can be much higher as a result of the leaching of zinc from piping and fittings.

The most corrosive waters are those of low pH, high carbon dioxide content, and low mineral salts content. Zinc is an essential trace element found in virtually all food and potable water in the form of salts or organic complexes. The diet is normally the principal source of zinc.

Although levels of zinc in surface water and groundwater normally do not exceed 0.01 and 0.05 mg/L, respectively, concentrations in tap water can be much higher as a result of dissolution of zinc from pipes. The 1958 WHO international standards for drinking water suggested that concentrations of zinc greater than 15 mg/L would markedly impair the potability of the water.

The 1963 and 1971 international standards retained this value as a maximum allowable or permissible concentration. In the first edition of the guidelines for drinking water quality, published in 1984, a guideline value of 5.0mg/L was established for zinc, based on taste considerations. The 1993 guidelines concluded that, taking into account recent studies on humans, the derivation of a guideline value was not required at this time. However, drinking-water containing zinc at levels above 3 mg/L may not be acceptable to consumers [63].

Other Inorganic Toxic Substances

Many toxic minerals are contained in water supplies, usually at high levels. Treatment plants work very well reducing these minerals to safe levels. Minerals can enter surface or ground water through natural sources, industrial sewage, and leakage from urban or agricultural areas, water pipes walls or even from domestic sources.

Aluminum: Aluminum is very abundant on the earth, but is not important for human nutrition. Aluminum can have toxic effects even in small quantities [64]. These effects occur in nervous system, but health effects originating from aluminum intake through water are still on debate. Aluminum concentration is usually lower than 200mg/L in drinking water. If you drink 1.5 liter of water per day, your daily intake from water is lower than 300mg/day, a negligible amount if compared with the amount taken by nutrition (10-20mg/day). There is no evidence that the aluminum consumed through water is more soluble and then more easily digestible, than the aluminum contained in food. Due to all these uncertainties at present there are no rules about its concentration allowed in drinking water. The WHO recommends a concentration lower than 20 mg/L.

Arsenic: Arsenic can be toxic even in low amounts. Nevertheless the arsenic contained in food (amounts ranging from 0.01 to 1.5mg/kg of dry weight) has a different influence: it carries out some positive metabolic function for our body. Its toxicity is strongly linked on the concentration [65].

Cadmium: Cadmium is a highly toxic heavy metal, considered carcinogen. Its harmful action is similar to the effect of lead and it can be released in drinking water by zinc and iron pipes. Zinc always contains a small amount of cadmium [65].

Lead: Lead is poisoning even in small amounts for microorganisms, interfering with hemoglobin formation and with the functionality of central nervous system. Lead is particularly harmful for children, who can suffer long term neurological and behavioral disorders. Major lead sources are paint, vehicle emissions, food and water. The WHO guideline about drinking water for human consumption states that the maximum allowed lead concentration in drinking water should not exceed 0.015mg/L and some precautions can be taken to lower lead content in drinking water [66].

Phosphate: In the body, phosphorus is combined with oxygen to form a variety of phosphates (PO_4^{3-}). Phosphates are vital for energy production, muscle and nerve function, and bone growth. They also play an important role as a buffer, helping to maintain the body's acid – base balance. About 70% to 80% of the phosphates are combined with calcium to help form bones and teeth, about 10% are found in muscle, and about 1% is in nerve tissue. The rest is found within cells throughout the body, where it is mainly used to store energy; about 1% of total body phosphate is found within plasma. Most phosphate in the body comes from dietary sources.

A variety of foods, such as beans, peas and nuts, cereals, dairy products, eggs, beef, chicken, and fish contain significant amounts of phosphate. The body maintains phosphate levels in the blood by regulating how much it absorbs from the intestines and how much it excretes or conserves in the kidneys. Phosphate in water originates from detergents and fertilizers and a level higher than 0.1 mg/L indicates pollution [67].

Sulphates: Sulphates are sulphuric acid salts combined with metallic ions. Water can naturally contains small quantities of sulfates, but they are mostly transferred in water bodies from the atmosphere and in the atmosphere from road traffic, industries and energetic production. Sulphur oxidized in the air can come back on the soil as acid rain causing serious environmental problems. Sulfate is a substance that is occurs naturally in drinking water.

Health concerns regarding sulfate in drinking water have been raised because of reports of diarrhea associated with the ingestion of water containing high levels of sulfate. Of particular concern are groups within the general population that may be at greater risk from the laxative effects of sulfate when they experience an abrupt change from drinking water with low sulfate concentrations to drinking water with high sulfate concentrations [68].

Nitrates and Nitrites: Nitrates are the main source of nitrogen for plants and an essential constituent for nucleic acids and amino acids. A nitrate water content of about 10 mg/L is considered normal and natural. Different concentration is due to human operations (mauling, air pollution due to transport). The problems resulting from excessive nitrate presence are due to the toxicity of nitrate for human body: nitrates are transformed in nitrites or in carcinogenic nitrosamines.

2.3.3 National and International Standards for Microbiological and Physico-Chemical Parameters

The intergovernmental body for the development of internationally recognized standards for food is the CAC. WHO, is one of the co-sponsors of the CAC, has advocated the use of the guidelines for drinking-water quality as the basis for derivation of standards for all bottled waters.

The CAC has developed a Codex Standard for bottled waters and an associated code of practice. The Codex Standard describes the product and its labeling, compositional and quality factors, including limits for certain chemicals, hygiene, packaging and labeling. The Codex Code of Practice for collecting, processing and marketing of bottled waters provides guidance to the industry on a range of good manufacturing practices matters.

While CAC standards and recommendations are not strictly mandatory, Codex health and safety requirements are recognized by the World Trade Organization as representing the international agreement for consumer protection and any deviation from Codex recommendations may require a scientifically-based justification.

Water is essential to sustain life, and a satisfactory (adequate, safe and accessible) supply must be available to all. Improving access to safe drinking-water can result in tangible benefits to health. Every effort then should be made to achieve drinking-water that is as safe and wholesome. Safe drinking water, as defined by the Guidelines, does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. In other direction, the nature and form of drinking-water standards may vary among countries and regions and there is no single approach that is universally applicable.

In the development and implementation of standards it is essential to consider the current or planned legislation relating to water, health and local government and the capacity of regulators in the country. Additionally approaches that may work in one country or region will not necessarily transfer to other countries or regions. For this work the CODEX and the adopted compulsory Ethiopian guidelines values for drinking water are presented in Table 2.4.

Table 2.4. Physical requirement for bottled water as per Ethiopian standard [69].

Characteristic	CES99			CES151	
	unit	Maximum permutable limit	Test method	Maximum permutable limit	Test method
General appearance	-	-	-	clear and free from any matter appreciable deposit	
Odor and taste		unobjectionable	ES605	unobjectionable	ES605
Turbidity ,max	NTU	5	ESISO7027	5	ESISO7027
Color max	TCU	15	ES ISO7887	15	ES ISO7887

Table 2.5. Characteristics that affect the palatability of bottled drinking water [69].

Substance or characteristic	CES99		CES151	
	Maximum permissible level	Test method	Maximum permissible level	Test Method
Total hardness (as $CaCO_3$) mg/I	300	ES 607	400	ES 607
Total dissolved solids mg/LMax	1000	ES 609	150-1500	ES 609
Total Iron (as Fe), mg/L Max	0.3	ES ISO 6332	0.3	ES ISO 6332
Manganese (as Mn) mg/L Max	0.5	ES ISO 6333	0.5	ES ISO 6333
Ammonia ($NH_3^+NH_4^+$)* mg/L, Max	1.5	ES ISO 7150-1	-	-
Magnesium (as Mg) mg/L, Max	50	ES ISO 7980	100	ES ISO 7980
Calcium (as Ca) mg/L Max	75	ES ISO 7980	200	ES ISO 7980
Copper (as Cu) mg/L Max	1	ES ISO 8288	1	ES ISO 8288
Zinc (as Zn) mg/L Max	5	ES ISO 8288	5.0	ES ISO 8288
Sulfate (as SO_4) mg/L Max	200	ES ISO 9280	200	ES ISO 9280
Chloride (as CI) mg/L Max	250	ES ISO 9297	200	ES ISO 9297
Total alkalinity (as $CaCO_3$) mg/L, Max	200	ES ISO 9963	75-600	ES ISO 9963-1
Sodium (as Na), mg/L, Max	200	ES ISO 9964	300	ES ISO 9964
pH value	6.0 to 8.5	ES ISO 10523	6.0-8.5	ES ISO 10523
Potassium (as K),mg/L, Max	50	ES ISO 9964-2	50	ES ISO 9964-2
Aluminum (as Al) mg/L Max	0.2	ES ISO 12020	0.2	ES ISO 12020

The term ammonia includes the non-ionized (NH_3) and ionized (NH_4) species.

Table 2.6. Content of toxic / disease causing substances of bottled drinking water [69].

Substance or characteristic	CES99		CES151	
	Maximum permissible level	Test method	Maximum permissible level	Test method
Barium (as Ba) mg/L, Max	0.7	ES 606	0.7	ES 606
Organic matter (as CO_2) mg/L Max	3	ES 608	3	ES 608
Total mercury (as Hg) mg/L, Max	0.001	ESISO5666-3	0.001	ESISO5666-3
Cadmium(as Cd mg/L, Max	0.1	ESISO5961	0.1	ESISO5961
Arsenic (as As) mg/L, Max	0.01	ES ISO6595	0.01	ES ISO6595
Cyanide (as CN^-) mg/L, Max	0.07	ES ISO6703-1	0.07	ES ISO6703-1
Nitrite (as NO_2) mg/L, Max	0.1	ES ISO6777	0.1	ES ISO6777
Nitrate (as NO_3)mg/L, Max	50	ES ISO7890-1,ESISO7890-2ESISO7890-3	45	ES ISO7890-1,ESISO7890-2ESISO7890-3
Chlorophenol mg/L, max	0.001	ESISO8165-1	0.001	ESISO8165-1
Pentaphenol mg/L, max	0.01		0.01	
2-4-6 Trichlorophenol mg/L, max	0.01		0.01	
Lead (as Pb) mg/L, Max	0.01	ES ISO8288	0.01	ES ISO8288
Boron mg/L, Max	0.3	ES ISO9390	0.3	ES ISO9390
Selenium (as Se) mg/L, Max	0.01	ES ISO9965	0.01	ES ISO9965
Fluoride (as F), mg/L Max	1	ES ISO10359-1	1	ES ISO10359-1
Chromium (as Cr) mg/L, max	0.05	ES ISO11083	0.05	ES ISO11083
Pesticides and organic constituents, µg/L, max				
DDT (O,P & P.P-isomers of DDT, ODE & DOD)	2	ESISO6468	2	ESISO6468
Heptachloro	0.03		0.03	
Heptachloroperoxide	0.03		0.03	
Hexachlorobenzene	1		1	
Lindane (Gamma- BHC)	2		2	
Methoxychlor	20		20	
Aldrin	0.03		0.03	
Dieldrine	0.03		0.03	
1,2 – dichloro ethane	30	ES ISO 10301 APHA 6232B*	30	ES ISO 10301 APHA 6232B*
1,1,1 – Trichloro ethane	2001		2001	
Trichloro ethene	70		70	
Trichlorobenzenes (total)	1		1	
Hexacholorobutadiene	0.6		0.6	
TrihalomethanePolynuclear aromatic hydrocarbons	Absent		Absent	
Polynuclear aromatic hydrocarbons	0.01	ES ISO 28540,ES ISO 17993	0.01	ES ISO 28540,ES ISO 17993

Table 2.7. Microbiological levels [69].

Organism	CES99		CES151	
	Maximum permissible level	Test method	Maximum permissible level	Test method
Coliform organisms, number per 100 ml	undetectable	ES ISO 9308-1, ES ISO 9308-2	undetectable	ES ISO 9308-1, ES ISO 9308-2
E. Coli, number per 100 ml	undetectable	ES ISO 9308-1, ES ISO 9308-2	undetectable	ES ISO 9308-1, ES ISO 9308-2
Total viable organisms, colonies per ml	100	ES ISO 4833	100	ES ISO 4833
Faecal streptococci per 100 ml	undetectable	ES ISO 7899-1 or ES ISO7899-2	undetectable	ES ISO 7899-1 /ES ISO 7899-2
Yeasts and molds	-		free	ESISO7954
Staphylococcus aureus per 100ml	Absent	ES ISO 6888-1, ES ISO 6888-2	Absent	ES ISO 6888-1, ES ISO 6888-2
Pseudomonas aeruginose per 100ml	Absent	ES ISO 16266	Absent	ES ISO 16266
Shigella per 100ml	Absent	ES ISO 21567, APHA 9260E*	Absent	ES ISO 21567, APHA 9260E*
Salmonella per 100ml	Absent	ES ISO 6579-2	Absent	ES ISO 6579-2

Table 2.8. CODEX standard of maximum contaminant levels in drinking water [69]

Substances	Maximum concentration level (mg/L)	Substances	Maximum concentration level (mg/L)
Antimony	0.005	Fluoride	1.5
Arsenic	0.01	Lead	0.01
Barium	0.7	Manganese	0.15
Borate	5	Mercury	0.001
Cadmium	0.003	Nickel	0.02
Chromium	0.05	Nitrate	50
Copper	1	Nitrite	0.02
Cyanide	0.07	Selenium	0.01

2.3.4 Sources of Contaminants in Drinking Water

In nature, all water contains some impurities. As water flows in streams, sits in lakes, and filters through layers of soil and rock in the ground, it dissolves and absorbs the substances that it touches. Some of these substances are harmless and required to humans but some are toxic. In fact, some people prefer mineral water precisely because minerals give it an appealing taste.

However, at certain levels of minerals, just like manmade chemicals, are considered contaminants that can make water unpleasant or even unsafe.

A number of chemical contaminants have been identified in drinking water. The chemical contaminants for which epidemiologic studies have suggested a risk associated with their presence in potable water include as discussed earlier includes: aluminum, arsenic, disinfection by-products (DBPs), fluoride, lead, nitrate, pesticides, cadmium, and mercury etc.

The contaminants are of both inorganic and organic origin. The source of the contaminant can be from point and non-point sources of pollution, naturally occurring, come from the treatment process or through materials used in distribution systems as seen in Figure 2.1 and 2.2 [69].

Naturally occurring contaminants are generally the result of leaching from geologic formations and are found primarily in groundwater. Ranges of concentrations of these contaminants range from less than Nano-grams per litter (ng/L) to milligrams per liter (mg/L). Point sources of drinking water contaminants include direct dumping of chemicals from domestic and industrial sewage. Other sources of pollution include runoff from land application of chemicals or leaching from buried solid waste landfills.

Finally, mining practices or smelter operations can increase the concentrations of metals in source waters through the atmospheric deposition or improper handling of mining tailings. The treatment process can be a significant source of chemical contaminants. Disinfectants themselves are not believed to be a significant health hazard at levels used to treat water for drinking.

The disinfectants (primarily chlorine or chlorine based), because of their strong oxidizing properties, react with the other organic constituents in the water to form chlorinated or brominated compounds believed to be of major toxicological concern. Aluminum and fluoride are both added to the treatment process but are not believed to be of concern at the levels they are added to water for treatment. It is when they are present as the result of geological leaching that concern has been raised.

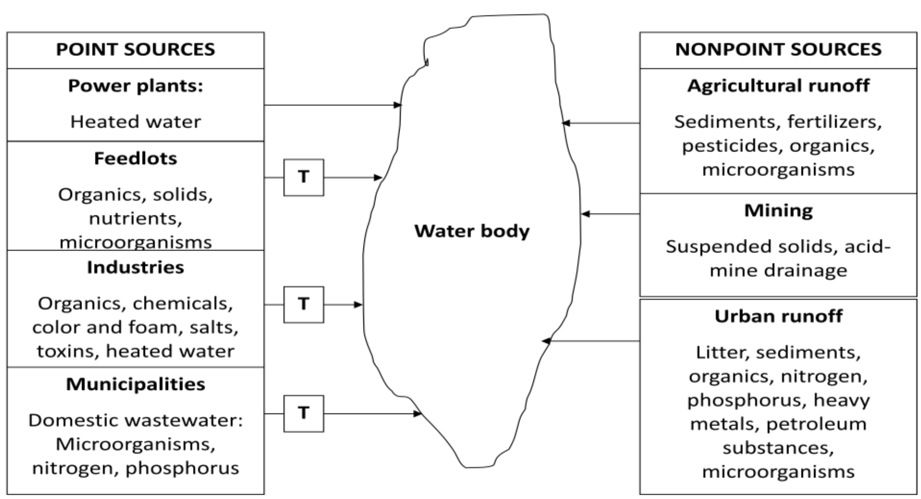

Figure 2.3. Sources of water pollution [56]

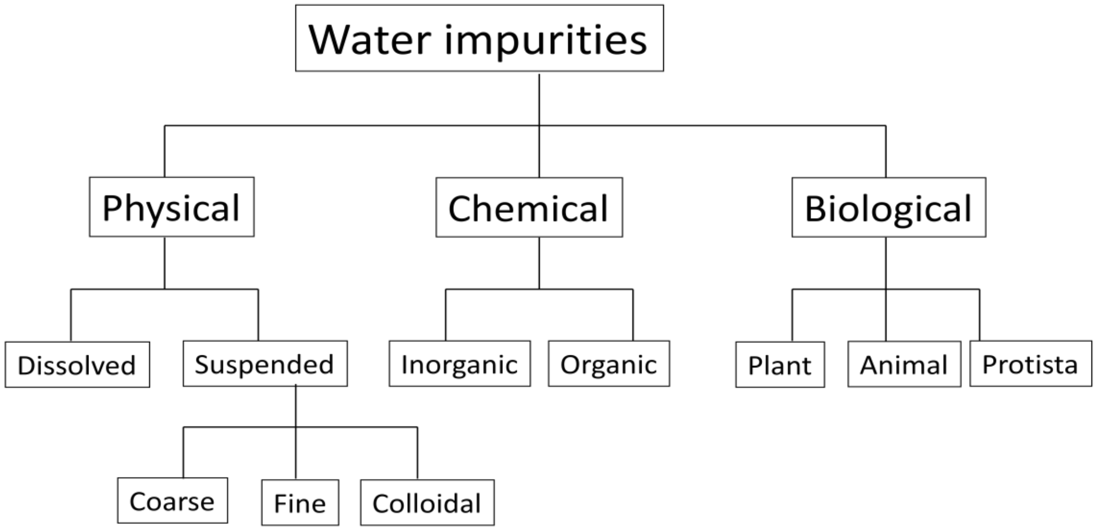

Figure 2.4. Water Impurities [56]

2.3.5 Health Effects of Contaminants in Drinking Water

a. Physical and chemical contaminants effect

The presence of physical and chemical contamination in groundwater consumed by human being performs great health risks. Dissolved substances (TDS) may be organic or inorganic and many of them are undesirable in water and produce displeasing color, taste and odors [70].

High contents of nitrogen forms (nitrate, nitrite and ammonia) in water reduce the oxygen carrying capacity of the blood [70]. Heavy metals accumulated in human body tissues, for example arsenic is accumulated in hair and nails, cadmium in kidney, mercury in hair and kidney and lead in bone and teethe [71]. Certain metals have been implicated carcinogenesis including chromium, nickel, cobalt, lead, iron, and cadmium [71].

Chemical contaminants occur in drinking water supplies throughout the United States, ranging from barely detectable amounts to levels that could possibly threaten human health. Determining the health effects of these contaminants is difficult, especially since researchers are still learning how chemicals react in the body to damage cells and cause illness [72]. As mentioned previously, concern with chemicals in drinking water started in outbreak situations where individuals became acutely ill. Chemical spills or leaks still occur causing acute like toxicity (primarily vomiting). As more chemicals could be found in potable water, studies began to appear in the literature linking health effects with occurrence of the contaminant of interest.

Cancer has been one of the more popular endpoints to study in relationship to effects associated with exposure to specific chemicals in water. Recent years have seen an interest in reproductive and developmental effects.

Studies of cancer and reproductive effects have been aided by the existence in many communities of databases of mortality or morbidity for these endpoints. The epidemiologic evidence in conjunction with toxicological data (human and animal) has been considered important in establishing causal relationships between the exposure and effects for arsenic, lead, nitrate, radon, etc. [64].

Toxic doses of chemicals cause either acute or chronic health effects. An acute effect usually follows a large dose of a chemical and occurs almost immediately. Examples of acute health effects are nausea, lung irritation, skin rash, vomiting, dizziness and even death. The levels of chemicals in drinking water, however, are seldom high enough to cause acute health effects. They are most likely to cause chronic health effects that occur after long exposure to small amounts of a chemical as can be seen in the tables below.

Examples of chronic health effects include cancer, birth defects, organ damage, disorders of the nervous system, and damage to the immune system as can be seen in Figures 2.5. Evidence relating chronic health effects to specific drinking water contaminants is limited. In the absence of exact scientific information, scientists predict the likely adverse effects of chemicals in drinking water using laboratory animal studies and, when available, human data from clinical reports and epidemiological studies [64].

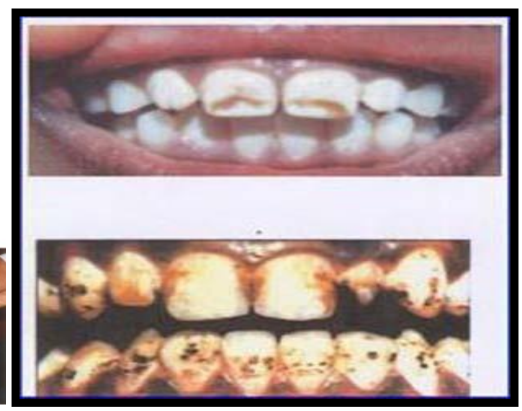

Figure 2.5. a. Skin lesions Figure 2.5. b mottled teeth

Figure 2.5. Health Effects of Heavy metals

Table 2.9. Summary of health effects of inorganic chemicals (Primary MCL)

Contaminants	Health effects	MCL (mg/L)
Arsenic	Nervous system effects	0.05
Asbestos	Possible cancer	7 MFL
Cyanide	Nervous system effects	0.2
Lead	Nervous system and kidney effects, toxic to infants	TT
Mercury	Kidney, nervous system effects	0.002

Table 2.10. Summary of health effects of organic chemicals (primary MCL)

Contaminants	Health effects	MCL (mg/L)
Benzene	Possible cancer	0.005
Total trihalomethanes (TTHM)	Possible cancer risk	0.1
Carbon tetrachloride	Possible cancer	0.005
Endrin	Nervous system, liver, kidney effects	0.002
Vinyl chloride	Possible cancer	0.002

Table 2.11. Secondary MCL

Contaminants	MCL (mg/L)	Health effects
Aluminum	0.05-0.2	Discoloration of water
Fluoride	2.0	Dental fluorosis (a brownish discoloration of the teeth)
Iron	0.3	Bitter metallic taste; staining of laundry, rusty color, sediment
Manganese	0.05	Taste; staining of laundry, black to brown color, black staining
Odor	3 TO	Rotten egg, musty, or chemical smell
Color	15 CU	Visible tint

b. Microbial Contaminants Effects

Water is a basic necessity for life. Unfortunately, not all water helps human to survive. Water from contaminated sources causes numerous diseases and untimely deaths.

The fact that a human needs water and cannot live without it forces him to use it even for drinking purposes, from any source, whether pure or contaminated.

Usage of quality deteriorated water may be a cause for the existence of water born, water washed water based and water related diseases. The term water associated disease is used to describe all infections whose causing agents are carried by water [74]. These are cholera, bacillary dysentery, Escherichia Coli (E.coli), viral hepatitis A, shigellosis, typhoid fever, cryptosporidiosis, giardiasis [74]. Generally, waterborne disease outbreaks usually involve, source contamination and the breakdown of the treatment systems, contamination of the distribution systems and the use of untreated water [74].

Water-associated disease is defined as a disease in relation to water supply and sanitation. There are four categories [56]. These are:

- Waterborne disease
- Water-washed disease
- Water-based disease
- Water-related disease

Waterborne Diseases:

Several infections enteric or intestinal diseases of man are transmitted through water contamination by fecal matter. Pathogens excreted in water by an infected person include all major categories such as bacteria, viruses, protozoa and parasitic warms. In this category water acts as a passive vehicle for the infectious agent. Some water born disease and their disease causing micro-organisms are presented. So if the bottled water is not treated properly the health of the consumers will be affected.

Table 2.12. Water borne diseases causing microorganisms

Diseases	Disease causing organism	Species	Source
Typhoid	Salmonellae typhus	Bacteria	Human feces
Cholera	Vibrio-cholera	Bacteria	Human feces
Amoebic dysentery	Entamoeba-histolitica	protozoa	Human feces
Giardiasis	Giardia-lamblia	protozoa	Human or animal feces

The presence of pathogenic bacteria in groundwater contributes a very dangerous health risks and diseases such as; cholera caused by *Vibrio chlera,* Typhoid fever, diarrhea and Salmonillosis caused by *S. typhi* and other *Salmonella* spp; *Shigellosis and* bacillary dysentery by *Shigella*, as well as, gastroenteritis by *E.coli* and *Campylobacter jejuni* [56]. About 70% of the waterborne microbial illness outbreaks in the United States have been associated with groundwater [74]. *Chryseobacterium meningosepticum* is ubiquitous Gram- negative rod historically associated with meningitis in premature neonates.

The authors reported 15 positive cultures and 6 cases of infection among immune-compromised adults. They found that there are 308 reports of positive cultures, of which 59% were determined to represent true infection. Sixty-five percent of those infected persons were younger than 3 months of age.

Meningitis was the most common infectious syndrome among neonates, seen in 84% of cases and associated with a 57% mortality rate. Less commonly reported infection among infants included sepsis (13%) and pneumonia (3%). Pneumonia was the most frequent infection among the post- neonatal group, accounting for 40% of cases, followed by sepsis (24%), meningitis (18%), endocarditic (3%), cellulites (3%), abdominal infections

(3%), eye infections (3%) and single case reports of sinusitis, bronchitis as well as epididymitis. The 6 cases in their report were all adults with a mean age of 58.7 years and Sites of *C. meningosepticum* infection were limited to the lungs, bloodstream and gall bladder.

Infection was associated with prolonged hospitalization, prior exposure to multiple antibiotics and host immune compromise, particularly neutropenia. An Outbreak of gastroenteritis affecting 730 students was seen in Taiwan in September 1993. *Shigella sonnei* and *Entamoeba histolytica* were isolated from the fecal specimens of patients.

Environmental investigations reveal the source of infection to be contamination of underground well water by sewage from toilet and reported 14 waterborne epidemics occurred in Finland during 1998-1999 and about 7300 illness cases were registered in these outbreaks; all were associated with nomadic infected groundwater [75].

The main reasons of groundwater outbreaks were floods and surface runoffs which contaminated the water. In most cases the outbreaks ceased by boiling the drinking water before use and starting chlorination. The world health organization has estimated that up to 80% of all sickness and diseases in the world is caused by inadequate sanitation polluted water or unavailable of water [76].

Fungi are widely distributed in nature and can occur as unicellular yeast or filamentous and, multi cellular molds. There are over thousands of species of fungi. Fewer than 300 have been implicated in human diseases, and fewer than a dozen cause about 90% of all fungus infections. They are involved in different forms of diseases, including allergies to fungal antigens, production of toxins, or direct invasion of hosts [77].

A one year fungal survey of a water bottling plant was conducted in order to evaluate the incidence and fluctuations of the biota. The dominant fungal genera in order of highest number were order *Penicillium*, *Cladosporium* and *Trichoderma* followed by *Aspergillus* and *Paecilomyces*. As expected, highest number of isolates collected was during the summer months, particularly May and June. Indeed during these two months there were more fungi present in the water after it had passed through the filtration system (0.4 micro meter filter), indicating that during those times of the year when fungal contamination is high, filters should be changed on a more regular basis.

In order to assess whether contamination was single or multi-cellular, molecular methods based on PCR were used. Overall fungal contamination arose from multiple sources. Some fungal strains were very "alike" and were detected during different sampling times, indicating that some strains were endemic to the plant. There was little evidence to suggest that fungi detected in the source water passed through to other parts of the plant. However, there was evidence that fungal strains isolated from the water filter were detected elsewhere in the factory, confirming the need to change filters more regularly during periods of high fungal contamination.

In order to improve quality control, a HACCP program was implemented and Best Practice Guidelines introduced [78]. Several species of fungi are capable of infecting healthy hosts and causing diseases ranging from mucosal to life-threatening disseminated infections. In addition, there is an increasing number of severe fungal diseases by commensally or fully saprophytic species in immune compromised hosts.

These diseases are frequently associated with abrogated host immunity as a result of viral infections, mainly the human immunodeficiency virus, hematological and hormonal disorders, organ transplants, antibiotic usage, and more intensive and aggressive medicals practices [79]. Fungal infections were difficult to treat since the agents were eukaryotes, as human cells. Despite their wide occurrence, little attention has been given to their presence and significance in aquatic environments. Drinking water distribution systems are colonized by saprophytic heterotrophic microorganisms (such as bacteria, fungi, yeast) that grow on biodegradable organic matter [80].

However, potentially pathogenic microorganism and microorganisms of fecal origin can also find favorable condition and proliferate in these systems.

The bacterial flora of bottled mineral water increases in numbers after bottling, reaching a peak by the end of one week [79]. After this time the bacterial count remains fairly constant for at least 6 months. The bacterial counts in still waters reach higher levels than in carbonated waters. There is some debate about whether bacterial counts reach higher levels in plastic compared with glass bottles. Several authors have found higher counts in waters stored in plastic or PVC bottles when compared with glass [81].

The reason put forward by these authors was that the inner surface of plastic bottles was rougher, so promoting adhesion and colonization. The quantity of bacteria in commercial mineral water is generally dependent of good manufacturing practices and autochthonous flora of the spring. It is well known that natural mineral water is characterized by its bacterial flora, chemical and physical composition. In addition to natural contamination, the product can also be deteriorated before it reaches the consumer [82].

The Brazilian directives regulate water from municipal water supplies on the basis of coliform content and heterotrophic plate count, whereas more stringent bottled mineral water regulations prohibit the presence of a group of potentially pathogenic bacteria (*Pseudomonas*, fecal streptococci and clostridia). Although microbiological standards exist for bottled water, the same product once installed on a dispenser is generally not regulated and is rarely controlled.

2.4 Overview of Water Treatment

Water is a very sensitive product on both microbiological and chemical levels. One of the main challenges of bottled water processing is to consistently produce a quality and safe product free of pathogenic organisms and protozoa that could contaminate its quality, reduce its shelf life, and are a pathogenic threat to customers. Strict production control is critical to avoid any contamination of pathogenic bacteria or protozoa.

While bottled water production processes differ by type of products and applications, the basic process is the similar. Arguably, it is possible to produce potable water from virtually any source of water. In practice, of course, there are a number of restrictions on the quality of water that can be treated. The usual reason for not using a particular source is cost but there are other reasons, notably ecological/environmental constraints and aesthetics, direct potable re-use of wastewater is usually unacceptable at present, although it has been practiced at Windhoek in Namibia since the late 1960s. Although ecological and environmental constraints can also be valued, aesthetic objections tend to have a value above mere money. There is a whole range of treatment processes that can be used to treat a particular type of water.

The actual processes selected must depend on the following treatment selection factors:

- Raw water quality/nature of raw water
- Availability and reliability of water supply
- Site suitability
- Land availability
- Proximity to consumers
- Economics
- Environmental impact assessment

There may not be a single correct process to treat particular water, but a number of possible options. But they can be categorized under three main classes like; traditional, modern; and the future.

2.5 Bottled Water Purification Technology

2.5.1 Brief history of filtration

Filters have been used to clarify water for thousands of years. Medical experiences written in India dating to 2000 BC, mentions filtration through sand and gravel was used as a method of purifying water.

Hippocrates advocated filtration through cloth bags in the fourth century BC. The Romans dug channels parallel to lakes to take advantage of natural filtration through soil when using lakes for water supplies. Venice, Italy, stored rainwater in cisterns but drew the fresh water from wells in sand that surrounded the cisterns [83].

The commercialization and patenting of filtration technologies started in France around 1750, using various filter media such as sponges, charcoal, wool, sand, crushed sandstone, or gravel.

The practice of filtering surface water through engineered systems and distributing it on a municipal scale began in England and Scotland around 1800. Various filtration concepts were tested, including flow direction (down flow, up flow, and horizontal flow), sand and gravel media graded from smaller to larger sizes, and backwashing by reverse flow. The first modern slow sand filter, designed by James Simpson for the Chelsea Water Works Company in London in 1829, incorporated an under drain system, graded gravel and sand media, a filtration rate of about 0.12 m/h (0.05gpm/ft^2), and cleaning by scraping and design features are still used today [83].

Filtration

Filtration is widely used for removing particles from water. Filtration can be defined as any process for the removal of solid particles from a suspension (a two-phase system containing particles in a fluid) by passage of the suspension through a porous medium. In granular filtration, the porous medium is a thick bed of granular material such as sand. Whether from a spring, borehole or surface water system, incoming water may have particles suspended or dissolved in it. Pathogens and Harmful chemicals from human activities (industrial wastes, pesticides, fertilizers and urbanization etc) should be removed to protect system and product quality. So, bottled water must be treated for physical, chemical and microbial contaminants.

For this purpose most water bottling companies in Ethiopia follow similar stages of purification. The water is processed with multi stage purification processes such as sand filter, activated carbon filter, ultraviolet disinfection, ultra filtration, and Reverse Osmosis and Ozone treatments.

Sand Filtration/ Multimedia filter

The multi-medium filter is built on mechanical filtration principle. It uses qualified quartz sand as filtration medium to effectively remove suspended solid, organism, colloid, bacteria, and so on by the following methods:

- Physical: Mechanical straining/sedimentation
- Chemical: Oxidation of organic matter by aerobic bacteria
- Biological: Occurs through *Schmutz decke* or “Vital layer”. *Schmutzdecke* is a layer of dirt, debris, and microorganisms build up on the top of the sand

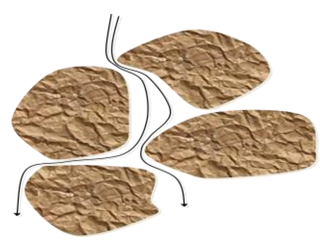

Figure 2.6. Particle removal mechanism in filters [83]

Activated Carbon Filtration

Once the water passes through the sand filtration system, it moves into granular activated carbon beds. Carbon filtration (also known as charcoal filtration), which utilizes a process known as adsorption, is a particularly effective technique for chlorine removal. Pesticides, herbicides, and other organic contaminants (especially volatile organics) are also removed at this stage. Adsorption is a mass transfer operation in which substances present in a liquid phase are adsorbed on a solid phase and thus removed from the liquid

Sediment Filtration

These filter functions are performed using polypropylene filters. The first filters remove sediment and other suspended particles. Trap filters remove particles that may be released by equipment, such as sand particles from the multi-media filter, carbon particle after the activated carbon vessel or particles from fractured deionizing or softening resin beads in the resin trap filter. Pre-filtration for the membrane system is also performed using depth filter media. Most systems manufacturers recommend removing particle larger than 5 microns to assure the life of the membranes. Some operators choose to remove even smaller particles to extend membrane life and reduce the need for system cleaning. The water then passes through another set of absolute filters. These being of the 1-Micron pore size, meaning that nothing larger than 1 Micron will pass through.

Ion Exchange/softening

This is the next step in the purification process of the removal of various metallic elements through a process known as ion exchange. Sometimes referred to as water “softening,” ion exchange utilizes a large tank which is filled with a special, negatively-charged resin. The resin beads serve as bases or sites for the ion exchange to actually take place. As water passes through the ion exchange resin, metallic ions, which carry a strong positive charge, displace the more weakly charged sodium and potassium ions. The metallic ions are thus trapped via electromagnetic attraction to the resin particles. The ion exchange beds are then automatically cleansed and regenerated at prescribed intervals based on water volume. The regeneration process involves flooding the bed with a super saturated saline solution that effectively sweeps away the metallic ions from the resin sites. Ion exchange provides highly effective removal of the metals responsible for pipe scaling and deposits and unpleasant taste in bottled water. The process also removes various heavy metals, such as lead, mercury, iron, and cadmium, which have been associated with well-publicized health concerns. It is important to remove metals early in the purification process to protect the more sensitive technology used in later steps, since high levels of metals can damage this equipment. Water softener is recommended when the hardness level of the water is greater than 150 ppm.

Table 2.13. Water Hardness Types

Carbonate (temporary) hardness causing compounds	Non-carbonate (permanent) hardness causing compounds
Calcium carbonate ($CaCO_3$)	Calcium sulfate ($CaSO_4$)
Magnesium carbonate ($MgCO_3$)	Magnesium sulfate ($MgSO_4$)
Calcium bicarbonate ($Ca(HCO_3)_2$)	Calcium chloride ($CaCl_2$)
Magnesium bicarbonate ($Mg(HCO_3)_2$)	Magnesium chloride ($MgCl_2$)
Calcium hydroxide ($Ca(OH)_2$)	
Magnesium hydroxide ($Mg(OH)_2$)	

Membrane Filtration

A membrane is a selective barrier that permits the separation of certain species in a fluid by combination of sieving and diffusion mechanisms and they can separate particles and molecules, over a wide particle size range and molecular weights [83].

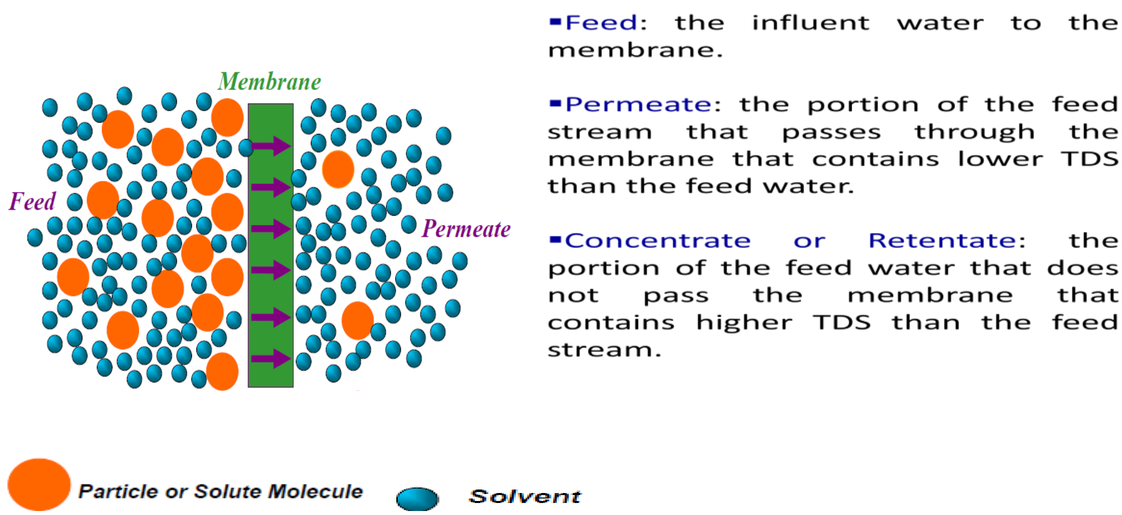

Figure 2.7. Schematic of membrane process

Membrane Process Types

There four main Pressure membrane processes used in water treatment process. **Microfiltration (MF)**: Membranes with a pore size of 0.1-10 µm perform microfiltration. Microfiltration membranes remove all bacteria. Only part of the viral contamination is caught up in the process, even though viruses are smaller than the pores of a microfiltration membrane. This is because viruses can attach themselves to bacterial bio-film. Microfiltration is implemented in many different water treatment processes when particles with a diameter greater than 0.1 mm need to be removed from a liquid.

Ultra-filtrations (UF): For complete removal of viruses, ultra filtration is required. The pores of ultra-filtration membranes can remove particles of 0.001 – 0.1 μm from fluids. UF provides consistent and reliable product water (typical turbidity < 0.10 NTU and SDI < 2) [83].

Figure 2.8. Vertical UF membranes Filtration

Nano-filtration (NF): Nano-filtration is a technique that has been prospered over the past few years. Today, Nano-filtration is mainly applied in drinking water purification process steps, such as water softening, discoloring and micro pollutant removal. During industrial processes Nano-filtration is applied for the removal of specific components, such as coloring agents. Nano-filtration is a pressure related process, during which separation takes place, based on molecule size. Membranes bring about the separation. The technique is mainly applied for the removal of organic substances, such as micro pollutants and multivalent ions. Nano-filtration membranes have a moderate retention for univalent salts.

Reverse Osmosis (RO): Two fluids containing different concentrations of dissolved solids that come in contact with each other will mix until the concentration is uniform. When these two fluids are separated by a semi permeable membrane (which lets the fluid flow through, while dissolved solids stay behind), a fluid containing a lower concentration will move through the membrane into the fluids containing a higher concentration of dissolved solids. After a while the water level will be higher on one side of the membrane. The difference in height is called the osmotic pressure. By pursuing pressure upon the fluid column, which exceeds the osmotic pressure, one will get a reversed effect. Fluids are pressed back through the membrane, while dissolved solids stay behind in the column. Using this technique, a larger part the salt content of the water can be removed. Reverse osmosis truly is the heart of the water purification process. Many people have heard about the process of osmosis. Osmosis is a naturally occurring process whereby water passes across a membrane due to a pressure differential between one side of the membrane and the other. During osmosis, the concentration of dissolved material on each side of the membrane moves closer to an equilibrium state. That is, the more concentrated solution will tend to become more diluted, and the more diluted solution will tend to become more concentrated. Many people are familiar with osmosis as the process by which living cells receive nutrients and excrete wastes. In reverse osmosis, high pressure is used to force water across a membrane while impurities are left behind. Table 2.14 shows the pressure requirement of different membrane types.

In other words, the high pressure causes the impurities to become more concentrated on one side of the membrane. Only the pure water is able to cross the membrane; even the dissolved impurities which cannot be removed by conventional filtration are captured and eliminated.

Electro dialysis (ED): electro Dialysis (ED) is a membrane process, during which ions are transported through semi permeable membrane, under the influence of an electric potential.

The membranes are cation or anion selective, which basically means that either positive ions or negative ions will flow through. Cation selective membranes are polyelectrolyte with negatively charged matter, which rejects negatively charged ions and allows positively charged ions to flow through. By placing multiple membranes in a row, which alternately allow positively or negatively charged ions to flow through, the ions can be removed from water.

This technique is not used for bottle water treatment except some sensitive health centers [83].

Table 2.14. Pressure requirement of membranes [83]

Membrane	Pore Size	Pressure
MF	Larger than UF	70 kPa
UF	0.001 – 10 µm	100 – 500 kPa
NF	Between UF and RO	500 – 1400 kPa
RO	0.0001 – 0.001 µm	1400 – 8300 kPa

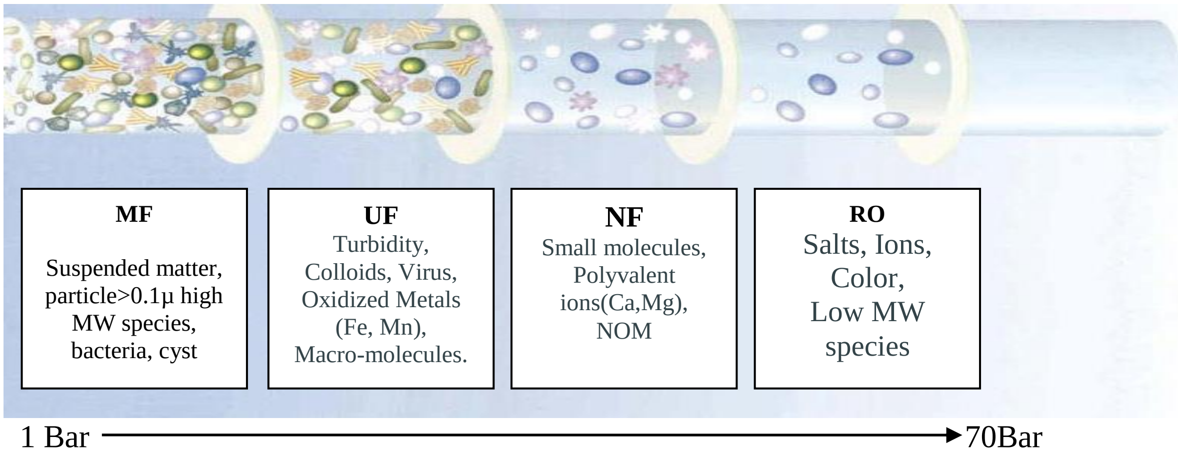

Figure 2.9. Filtration Spectrum

Figure 2.10. RO membranes with FRP vessel

Ultraviolet Light

Ultraviolet disinfection is the first of these technologies. At this step in the process, the water passes through a special chamber which houses a large ultraviolet light source. This ultraviolet light acts as a powerful sterilizing agent. If any bacteria, viruses, or other microbiological contaminants are present in the water, the ultraviolet light at this particular wavelength destroys the genetic material within these organisms, eliminating the possibility of bacterial or viral reproduction and proliferation. The organisms quickly die and are captured and removed during the pre-filtering before the membrane purification process. Radiation with a wavelength of around 260 nm penetrates the cell wall and cell membrane of microorganisms and is absorbed by cell material such as DNA and RNA and promotes changes that prevent replication to occur.

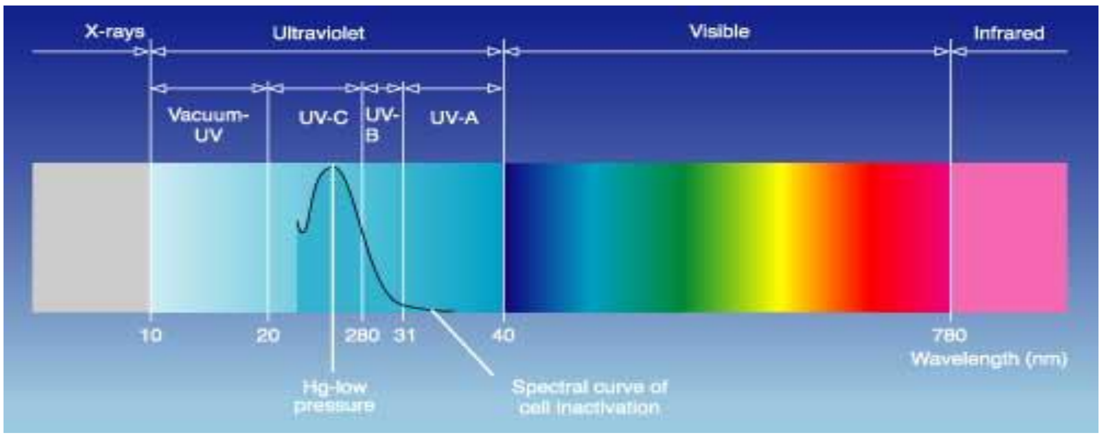

Figure 2.11. Wavelength

Figure 2.12. UV Disinfection apparatus

The main advantages of the UV disinfection over chlorine disinfection is that there is no disinfection by-products, short detention times (six-to-10-second contact time) compared to a 15-to-30-minute contact time for chlorine. UV disinfection presents no dangers to humans.

Ozone Treatment

The most important part of the chemical-free disinfection is known as ozone treatment. Ozone treatment relies on oxygen to ensure that our purified water remains free of any possible microbiological contamination. The ozone treatment process takes basic molecular oxygen (O_2) and passes it through a special chamber in which it is exposed to a high electrical voltage charge. This type of ozone generation is a system called cold-plasma discharge. The electricity causes the oxygen molecule to split and recombine in a higher-energy form known as ozone (O_3). This ozone is then continuously circulated through the purified water.

Ozone is a very powerful disinfectant and is capable of oxidizing a very broad range of contaminants. In fact, ozone is highly effective against many types of impurities and organisms, such as cryptosporidium, that are completely impervious to chlorination. Ozone is about 1,500 times more effective than chlorine as an oxidant. In real life, ozone is very effective at killing cryptosporidium. Tests have shown that at normal concentration levels (1 part per million), ozone will destroy 99.99% of cryptosporidium cysts given five minutes of contact time. Ozone is not a stable state form of oxygen, and over the course of a few minutes it returns to its natural O_2 state.

Figure 2.13. Ozone generators with contacting tower

Currently, in our country many water bottling companies are using reverse osmosis, some ultra-filtration and very few Nano-filtration systems in addition to the sand, carbon and cartridge filters. The use of the RO makes the water to be de-mineralized. Many people like the Demineralized water as it has lighter taste than the mineralized one. But the WHO report on Demineralized water declares waters less than 50 mg/l TDS are not recommended for drinking .So the use of RO and drinking water with a very low TDS level or Demineralized one for long time has health impact .On top of this there are many companies label their bottled water with a very low TDS /mineral content Natural mineral water which contradicts the standard.

Advantages and Disadvantages of Membrane Filtrations

Microfiltration:

Advantages

- It removes sand, silt, clays, Giardia lamblia and Crypotosporidium cysts, algae, and some bacterial species
- Another application for the technology is for removal of natural synthetic organic matter to reduce fouling potential
- It can be used as a pretreatment to RO and NF to reduce fouling potential

Disadvantages

- It is not an absolute barrier to viruses
- It removes little or no organic matter
- It removes only coarse particles

Ultra-Filtration

Advantages

- No need for chemicals (coagulants, flocculants, disinfectants, pH adjustment)
- Constant quality of the treated water in terms of particle and microbial removal
- Process and plant compactness
- These membranes are available in various sizes, making the process suitable for many different separations
- Because it separates without phase change, energy consumption is low, varying with the application

- The separation and concentration can be performed at low pressures, conversions can be high, colloids can be effectively removed, and macromolecular species can be concentrated
- It is particularly suitable to applications involving temperature-sensitive materials
- It systems are simple and compact
- It effectively removes most particles, pyrogens, microorganisms, and colloids above their rated size
- Produces highest quality water for least amount of energy

Disadvantages

- It cannot separate dissolved salts or low molecular weight species
- It cannot remove dissolved inorganics

NANOFILTRATION

Advantages

- It can remove virtually all cysts, bacteria, viruses, and humic materials
- It provide excellent protection from DBP formation
- It keeps the minerals as they are

Disadvantages

- It requires a higher operation pressure than either MF or UF

- It removes alkalinity, the product water can be corrosive,
- It removes hardness from water, which accounts for NF membranes sometimes being called "softening membranes." Hard water treated by NF will need pretreatment to avoid precipitation of hardness ions on the membrane.

Reverse osmosis

Advantages:

- It effectively removes all types of contaminants to some extent (particles, pyrogens, microorganisms, colloids and dissolved inorganics)
- It requires minimal maintenance
- It removes nearly all contaminant ions and most dissolved non-ions
- Low effluent concentration possible
- Bacteria and particles are also removed
- Operational simplicity and automation allow for less operator attention and make RO suitable for small system applications

Disadvantages

- Flow rates are usually limited to a certain gallons/day rating
- High capital and operating costs
- Managing the wastewater (brine solution) is a potential problem
- High level of pretreatment is required in some cases
- Membranes are prone to fouling
- It removes healthy minerals from drinking water

- Produces permeate between 25-50 percent of the feed [84].

2.6 Bottling Process

The first step for setting up a water purification plant is the analysis of source of water. After the chemical analysis, the specifications of the purification plant are set. In the purification plant, source water is stored in the feed water tank, passes through the sand filter for preliminary water filtration. Water then passes through a dosing pump and the carbon filter. It helps in the maintenance of proper odor and taste of the water. It also removes chlorine from water. Water is then passes through another dosing pump, through membrane module system. This stage of the process makes water clear from all the contaminations and minute particles. Water then passes through dosing pump, where minerals are added for taste development. After this stage, water undergoes Ultra Violet treatment to avoid any contamination from bacteria and other microorganisms. Water then passes through automatic washing, filling and capping plant. Here water is filled into bottles. After filling bottles are taken into the warehouse or shipped to the retailers. The complete process flow diagram is seen in figure 2.14 [83].

A water treatment process is custom designed for the type of water which needs to be processed to bottled water. During bottled water processing, water is collected from the natural spring, bore hole or any other source. All possible gasses (if any), iron, manganese and other unwanted minerals are removed. Depending on the raw water quality, air under pressure is injected into the water; pressured water and air react for ten minutes. This process oxidizes unwanted metals in the water. The water with oxidized metals is sprayed in a buffer tank with a holding capacity of approximately 4 hours. This spraying process removes unwanted gases out of the water. The first stage treated water is then pumped through a back wash Micro filter, which removes all unwanted sediment and oxidized metals. After the sediment removal filter, a titanium filter follows, which takes care of all possible left over dissolved metals [83].

The product water is now free of all exceeding dissolved metals and gasses and is chemically ready to be bottled. It is pumped through a 5 micron filter and a UV disinfection unit to a holding tank which needs to be at least one day of production capacity. This tank needs to be built in SS316 metal quality. The water is then pumped out of the holding tank trough an Ozone disinfection unit and 1 micron high purity filter to the bottling line. The bottled water needs to have 2-5ppm ozone in the water before capping the bottle [17].

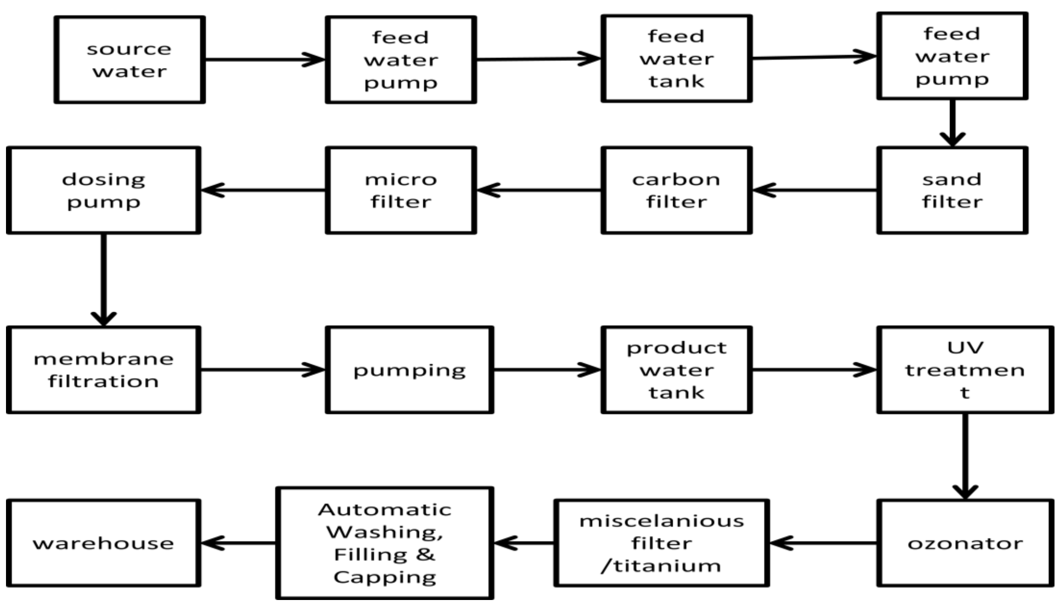

Figure 2.14. Water bottling Flow Diagram [83]

Figure 2.15. Partial view water bottling line

2.7 Bottled Waters Consumed in Addis Ababa

Addis Ababa as the capital city of Ethiopia and Africa with a population over 4.4 million, head quartered the African Union and the UN Economic Commission of Africa hosts numerous international conferences [85, 86]. These all making Addis Ababa an important center.

There are also large numbers of hotels, restaurants, recreational place for wedding ceremonies and for other purposes. So for this reason, safe and standardized drinking and bottled waters are required to satisfy both needs. In this capital city of Ethiopia different brands of locally produced bottled waters both purified and mineral waters are available in the local market. These bottled waters are used by many Ethiopians and tourists visiting Ethiopia for satisfying their water requirement and enjoyment purposes. Currently there are about 67 different brands of waters for purified, natural mineral and carbonated water types. Some of these known bottled waters are stated in table 2.15.

Advertisement for bottled waters in Addis Ababa shows bottled water is better than drinking tap water. Irrespective of the standard requirement (CES99 and CES151), they declare natural mineral water benefits, while they are purified, some of them call themselves organic, though there is no organic water etc. However, the regulations on the bottled waters may looks relaxed; the actual product versus the labeling and naming, safety and quality and customer awareness may all have problems. Hence there is room that anyone can arbitrary name his/her water as natural spring water or mineral water and some say even organic without fulfilling requirements and sell deliver it to the market without even conducting any analysis and it is likely to be contaminated [16].

Therefore, it is important to determine and compare the content of the bottled waters consumed in Addis Ababa with that of worldwide standards in general and the ions listed on labels of bottled water in particular. In this particular research the concentration of selected pollutants, cations and anions and microbial parameters in six brands of the most common commercial bottled drinking water (A,B,C,D,E,F) are presented. This research is carried out to clarify some of the concerns about the quality and safety of bottled drinking water which practically costs much higher than public drinking water.

Table 2.15. List of some water Bottling companies [87]

S/n	Brand name	Product Label	Manufacturing areas
1	Abyssinia spring	Natural spring water	Sululta oromia
2	Promise spring	Natural mineral water	Sebeta oromia
3	Pacific water	Natural drinking water	Menagesha oromia
4	Aqu addis	Natural spring water	Burayu oromia
5	Arki	Rich in natural mineral	Sululta oromia
6	Eden	Organic spring water	Agena ,welkite snnp
7	Cheers	Mineral water	Garamoye chancho oromia
8	One	Natural mineral water	sebeta oromia
9	Ava	Natural mineral water	Burayu oromia
10	Diamond	Natural mineral water	Ashewa meda oromia
11	Aqua nova	Natural mineral water	Sebeta oromia
12	Origin	Purified natural mineral water	Alemgena oromia
13	Fham	Pure mineral water	Tatek,gefersa nono oromia
14	Selam	Natural spring water	Shashemene,oromia
15	Aqu safe	Pure natural spring water	Debre brehan ,amhara
16	Classy	Natural purified water	Sululta oromia
17	Bekoji	Natural spring water	Bekoji ,oromia
18	Dasani		Nairobi bottlers ltd
19	Gift	Purified natural water	Sululta oromia
20	Sheger	Natural mineral water	Alemgena, sebeta oromia
21	Uniqe	Natural mineral water	Alemgena ,sebeta oromia
22	Yes	Natural mineral water	Alemgena ,sebeta oromia
23	Real spring	Natural spring water	Samit addis ababa
24	Kool	Natural mineral water	
25	Ambassador	Purified natural water	
26	Prima aqua	Natural mineral water	Akaki addis ababa
27	Agmas spring	Purified bottled water	ayer tena addis ababa
28	Blu	Natural mineral water	Holeta oromia
29	Wow	Natural mineral water	Gurage,snnp

2.8 Labeling Requirements

Our country has compulsory standards for both bottled and bottled natural mineral waters. The labeling standard is a compulsory standard that must be fulfilled.

The standard states the following issues:

- Applied labels on bottles shall be clean, neat and securely attached.
- They shall not be super imposed on other labels or on printed matter printed direct on the bottles.
- They shall not be applied by any person other than the manufacturer or his authorized agent.
- And regarding the name of the product the standard states the name of the product shall be a true description of the product concerned.
- The product must use the standard logo with either CES99, for bottled water or CES151 for bottled natural mineral water
- The composition of the product shall be declared in advertising and on the label, the concentration, in units of mg/l, of the following constituents being included in the declaration and listed in the following order: Ca, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}, $CaCO_3$,NO_3^{-},F^{-},Fe^{+3},Bicarbonate as $CaCO_3$,TDS and pH in mg/l except for pH. [17]

Chapter 3: Materials and Methods

3.1 Materials and Reagents

The following are the equipments and materials used during the laboratory work.

Equipments:

- Spectrophotometer lovibond MD600/Maxi Direct
- AD 131 and ADWA AD1020 meters for pH/Mv/Temperature /ISE
- AF330 EC/TDS meter
- Compound Microscope
- Incubator to grow the microbes
- Colony counter
- Bio-safety hood
- Auto clave
- Oven
- Hotplate
- Balance
- Petri dishes
- Test tubes and others
- Distiller

Chemicals:

Chemicals like pH buffers , Acid/Indicator, Eriochrome, Cyanine, Indophenol blue, Silver nitrate tablets, DPD, SPADNS reagent, several types of medias and reagents for microbial growth, Metallphthalein, Ferrozine, Ammoniummolybdate, Bariumsulfate, HNO_3 (69- 72%), deionized water, and Stock solutions HNO_3 were used.

3.2 Description of the sampling area

Addis Ababa, the capital city of Ethiopia, lies at about 7,546 feet (2,300 meters) above sea level ,with a total area of about 527 square kilometers and is a grassland biome located at $9^{o}1^{'}48^{''}$N $38^{o}44^{'}24^{''}$E [86,87]. The city has a subtropical highland climate and possesses a complex mix of highland climate zones, with temperature differences of up to 10^{0}C, depending on the elevation and prevailing wind patterns. The high elevation moderates temperatures year-round, and the city's position near the equator means that temperatures are very constant from month to month.

As the population of Addis Ababa is increasing the city has faced shortage of clean drinking water and occupied by people having different wealth status, there is huge market potential for bottled waters both for the mineralized and purified waters.

As a result almost all bottling companies are established in and nearby Oromiya special zones, Debrebirhan area, Dessie areas and Gurage areas, so there is access of getting almost all water types consumed in the city. The sampling sites were covered both directions and conducted at different locations of the city from supermarkets and shops which were selected randomly.

Currently, there are about 67 bottling companies certified by the Ethiopian Conformity Assessment enterprise and sell their products in the Ethiopian market; out of these about 40 sell their products in Addis Ababa city market [87].

3.3 Study design/Methods

For the completion of the study, statistical analysis of the obtained data from the bottled waters analysis, a correlation was developed between the parameters by applying Karl Pearson's coefficient of correlation. MS Excel was used to find out the mean, standard deviation (SD) and median of the data. Descriptive study design was applied to examine the standards available for bottled waters from the national and international standards, to know the treatment methods and facilities used by the selected companies and the advantages and disadvantages of the system; a site visit and literature review was conducted. Experimental study design was used for assessing physicochemical and bacteriological quality of the bottled drinking waters.

3.4 Sample collection and preparation

Eighteen bottles representing six most popular brands of bottled drinking water samples (3 for packaged natural mineral waters as per their label, A, B and E and 3 for bottled waters C, D and F similarly) were selected and collected randomly from different supermarkets around Megenagna (shewa supermarket) and Bole (Shewa and Fresh corner) in Addis Ababa, Ethiopia starting from June to July, 2017.

The collected samples were sealed in plastic bottles with production and best before dates which were stored in cold containers away from sunlight and heat and the selected bottling companies' transportation system is in closed vans. The products of the six brands were transported in closed vans protected from sunlight and heat from factory to retail supermarkets. To keep the names of brand anonymous, the samples were coded from A to F (A, B, C, D, E, and F). Eighteen bottles of water samples from each brand were used to analyze the selected parameters- pH, Electrical Conductivity (EC), Total Dissolved Solid (TDS), Total Hardness (TH), Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, Zn^{2+} and Cl^-,SO_4^{2-} ,NO_3^-, PO_4^{3-}, F^- and bacteriological identification and numeration. The collected bottled water samples were transported to the MGF Industries PLC and ECA laboratories for analysis at different times.

All samples were analyzed within about hours of collection to avoid the growth or death of organisms in the samples; each bottle was properly sealed and aseptically opened so that samples of water were analyzed properly. The collected samples production dates were two months during collection.

3.5 Experimental setup and procedure

3.5.1 Physico-chemical Analysis

All the equipments used for the test were calibrated by the Ethiopian Metrology Agency and by internal chemicals calibration.

Determination of pH:

The pH of the sample waters were tested by using pH meter (model: ADWA, AD1020) equipment (Figure 3.1a).The equipment was calibrated by using pH 4.01 during measuring acidic range and 10.01 buffers for alkaline range. After making sure the calibration by pressing the range button, selecting pH and then immersing the pH electrode tip and the temperature probe approximately to 4cm in the water sample and by allowing the electrode to be stabilized and read the values of the temperature and the pH is displayed in the LCD display.

Determination of EC and TDS:

AD330 EC/TDS digital meter was used to measure the TDS and EC of the sample waters by simply pressing the on/off switch; manually selected a range of the sample waters pH and adjusted and fixed the temperature probe. Then by rinsing the pH probe with distilled water, dipping the electrode in the sample water and stirring gently. After waiting until the reading stops flashing, this means the reading is stable. Then by the use of the MODE key selected both TDS and EC turn by turn then the conductivity and TDS readings were recorded.

Determination of Turbidity:

Turbidity was measured by direct reading using the MD600/ Maxi direct Spectrometer in the range of 0 – 1000 FAU. Filling a vial with a 24 mm in diameter with 10 ml of de-ionized water and pressing 0 to zeroing. And then by removing the vial from the sample chamber and empty it completely. Stirring the water sample, rinse the vial with the water sample and fill with 10 ml of water sample, swirling gently several times and by placing it in the sample chamber making sure that the marks on the vial and the instrument are aligned pressing test button it gave the result and recorded.

Determination of Color (true and apparent):

This was tested by using the platinum-cobalt standard method and was measured by direct reading by the use of the MD600/ Maxi direct Spectrometer as per the following procedure.

Sample preparation:

Step A

Filtering 50 ml of de-ionized water through a membrane filter with a pore width of 0.45 μm and discarding the filtrate and add new 50 ml de-ionized water was filtered and kept it for zeroing.

Step B

50 ml water sample was filtered using the same filter and this filtrate was kept for sample measurement. Then:

1. A clean vial (24 mm in diameter) was filled with 10 ml of the filtrated de-ionized water (from Step A), and closed tightly with the cap.
2. The vial was placed in the sample chamber by making sure that the marks on the vial and instrument are aligned.
3. Pressing ZERO key and removed the vial from the sample chamber and emptied it completely.
4. Rinsed the vial with the filtrated water sample and filled with 10 ml filtrated water sample (from Step B).
5. By placing the vial in the sample chamber, making sure that the marks are aligned, then pressing TEST key. The result was shown in the display in Pt-Co units.

Determination of Chloride (Cl^-):

Chloride was measured by using MD 600/Maxi Direct photometer using silver nitrate tablet within the range of 0.5 – 25 mg/l.

Procedure:

A clean vial (24 mm Ø) was filled with 10 ml of the water sample, and closed tightly with the cap and placed it in the sample chamber making sure that the marks are aligned. Then by pressing the ZERO key for zeroing and removed it from the sample chamber.

1. One CHLORIDE T1 tablet was added straight from the foil to the water sample, after crushing it using a clean stirring rod and dissolved it.
2. One CHLORIDE T2 additional tablet was added straight from the foil to the same water sample and crushed using a clean stirring rod.
3. The vial was tightly closed with the cap and swirled gently several times until the tablet is dissolved .Then the vial was placed in the sample chamber by making sure that the marks on the vial and instrument are aligned.
4. Then by pressing the TEST key and waiting for a reaction period of 2 minutes the measurement started automatically. The result was shown in the display in mg/l Chloride.

Determination of Total Hardness:

The TH was determined by using the HARDCHECK P tablet as can be seen in the steps below.

Step I: Hardness Range from 2 – 50 mg/l $CaCO_3$

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and closed tightly with the cap. This was placed in the in the sample chamber by aligning the marks on the vial and instrument. Then the by pressing the ZERO key, the vial was removed from the sample chamber.

2. One HARDCHECK P tablet was added straight from the foil to the water sample and crushed it using a clean stirring rod. Then by closeting the vial tightly with the cap and swirl several times until the tablet is dissolved; was placed it in the sample chamber again.
3. Finally by pressing the TEST key and waiting for a reaction period of 5 minutes, the measurement starts automatically. The result was shown in the display total Hardness in mg/l as $CaCO_3$.

Step II. Total Hardness ranging 20 – 500 mg/l $CaCO_3$

1. A clean vial of 24 mm in diameter was filled with 1 ml of the water sample and 9ml of the de-ionized water and closed tightly with the cap. This was placed in the in the sample chamber by aligning the marks on the vial and instrument. Then the by pressing the ZERO key, the vial was removed from the sample chamber.
2. One HARDCHECK P tablet was added straight from the foil to the water sample and crushed it using a clean stirring rod. Then by closeting the vial tightly with the cap and swirl several times until the tablet is dissolved; was placed it in the sample chamber again.
3. Finally by pressing the TEST key and waiting for a reaction period of 5 minutes, the measurement starts automatically. The result was shown in the display total Hardness in mg/l as $CaCO_3$.

The anions like SO_4^{-2}, NO_3^-, PO_4^{3-}, $F^-$and cations such as K^+, Fe^{2+}, Zn^{2+} were determined by UV spectrophotometer (Model: ADWA MD600/Maxi Direct) by using different reagents and methods as can be seen in the procedures below .

Determination of SO_4^{-2}

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and closed tightly with the cap. This was placed in the in the sample chamber by aligning the marks on the vial and instrument. Then the by pressing the ZERO key, the vial was removed from the sample chamber.
2. One **SULFATE T** tablet was added straight from the foil to the water sample and crushed it using a clean stirring rod. Then by closeting the vial tightly with the cap and swirl several times until the tablet is dissolved; was placed it in the sample chamber again.
3. Finally by pressing the TEST key and waiting for a reaction period of 2 minutes, the measurement starts automatically. The result was shown in the display in mg/l sulfate.

Determination of NO_3^-

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and closed tightly with the cap. This was placed in the in the sample chamber by aligning the marks on the vial and instrument. Then the by pressing the ZERO key, the vial was removed from the sample chamber emptied it.
2. The Nitrate test tube was filled with 20 ml of the water sample and 1 level spoon of Nitrate test powder was added. By tightly closing the tube with the cap, was swirled vigorously for one minute.
3. One NITRATE TEST tablet was Add straight from the foil to the water sample and closing the tube tightly with the cap and swirled vigorously for one minute.

4. By standing the tube upright and after the reducing agent has settled to the bottom, gently inverted it four times so as to complete the flocculation of the reducing agent. Then let the tube stand for a further 2 minutes. Open the tube and wipe around the top of the tube with a clean tissue to remove any residuals of the reducing agent.

5. Carefully decanted the 10 ml of the treated solution into the vial (24 mm in dia.) used for zeroing, ensuring that no reducing agent is carried over.

6. One NITRITE LR tablet was added straight from the foil to the water sample and crushed it using a clean stirring rod and by tightly closing with the cap, swirled it several times until the tablet is dissolved.

7. Placing the vial in the sample chamber making sure that the marks are aligned then press TEST key, wait for a reaction period of 10 minutes. After the reaction period is finished the measurement starts automatically. The result was shown in the display in mg/l Nitrate.

Determination of PO_4^{3-}

1. Inserting the adapter for 16 mm diameter vial.
2. Opening the cap of one digestion tube by PO_4-P Acid reagent and adding 5 ml of the water sample.
3. Adding the contents of one Vario Potassium Persulfate F10 Powder Pack straight from the foil to the vial.
4. Closing the vial tightly with the cap and inverting several times to mix the contents.
5. Heating the vials for 30 minutes in the preheated reactor at a temperature of 100°C.

6. After 30 minutes removing the vial from the reactor and taking care of the hot vial and allow the vial to cool to room temperature.
7. Opening the cooled digestion vial and adding 2 ml 1.54 N Sodium Hydroxide Solution to the vial.
8. Closing the vial with the cap and inverted it gently several times to mix the contents.
9. Placing the vial in the sample chamber making sure that the marks are aligned.
10. Pressing ZERO key.
11. Removing the vial from the sample chamber.
12. Adding the contents of one VARIO Phosphate Reagent F10 Powder Pack straight from the foil to the vial.
13. Closing the vial tightly with the cap and swirl several times to mix the contents (approx. 10-15 sec).
14. Placing the vial in the sample chamber and making sure that the marks are aligned.
15. By pressing TEST key and waiting for a reaction period of 2 minutes. After the reaction period is finished the measurement starts automatically and the result was shown in the display in mg/l total Phosphate.

Determination of F^-

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and closed tightly with the cap. This was placed in the sample chamber by aligning the marks on the vial and instrument. Then by pressing the ZERO key, the vial was removed from the sample chamber.

2. Exactly 2 ml SPADNS reagent solution was added to the water sample and vial was filled up to the top
3. By closing the vial tightly with the cap and swirl several times to mix the contents and was placed in the sample chamber making sure that the marks are aligned.
4. By pressing TEST key. The result was shown in the display in mg/l Fluoride.

Determination of K^+

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and closed tightly with the cap. This was placed in the sample chamber by aligning the marks on the vial and instrument. Then the by pressing the ZERO key, the vial was removed from the sample chamber
2. One Potassium T tablet was added straight from the foil to the water sample and crushed it using a clean stirring rod.
3. By closing the vial tightly with the cap and swirl several times until the tablet is dissolved and was placed in the sample chamber making sure that the marks are aligned.
4. By pressing TEST key and the result was shown in the display in mg/l Potassium.

Determination of total Fe

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and closed tightly with the cap. This was placed in the sample chamber by aligning the marks on the vial and instrument. Then the by pressing the ZERO key, the vial was removed from the sample chamber

2. The contents of one Vario Ferro F10 Powder Pack was added straight from the foil to the water sample and stirred it using a clean stirring rod.
3. By closing the vial tightly with the cap and swirl several times to mix the contents and was placed in the sample chamber making sure that the marks are aligned.
4. By pressing the TEST key and waiting for 3 minutes, the result was shown in the display in mg/l iron.

Determination of Zn^{2+}

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and one COPPER / ZINC LR tablet was added straight from the foil to the water sample, by crushing it using a clean stirring rod.
2. By closing the vial tightly with the cap and swirl several times until the tablet is dissolved and was placed in the sample chamber making sure that the marks are aligned.
3. By pressing TEST key and waiting for a reaction period of 5 minutes the measurement started automatically.
5. Removed the vial from the sample chamber.
6. One EDTA tablet was added straight from the foil to the prepared vial and crushed the tablet using a clean stirring rod.
7. By closing the vial tightly with the cap and swirled several times until the tablet was dissolved. The vial was put in the sample chamber making sure that the marks are aligned.
8. Pressing TEST key and the result was shown in the display in mg/l Zinc.

Determination of Ca^{2+}

1. A clean vial of 24 mm in diameter was filled with 10 ml of the water sample and closed tightly with the cap. This was placed in the sample chamber by aligning the marks on the vial and instrument. Then by pressing the ZERO key, the vial was removed from the sample chamber
2. One CALCIO H No. 1 tablet was added straight from the foil to the 10 ml water sample, crushing the tablet using a clean stirring rod and dissolved the tablet completely and one CALCIO H No. 2 tablet was added straight from the foil to the same water sample and crushed it using a clean stirring rod.
3. Closing the vial tightly with the cap and swirl gently several times until the tablet was completely dissolved and it was placed in the sample chamber making sure that the marks are aligned.
4. Finally by pressing TEST key and waiting it for a reaction period of 2 minutes till the reaction period was finished then measurement started automatically. The result was shown in the display as Calcium Hardness.

Determination of Mg^{2+}

The concentrations of the magnesium ions were determined by titration method.

Determination of Na^{+}

Sodium-ion selective electrode method was used to determine it.

Equipment used

1. AD1020 Digital pH/mV meter
2. Ion Selective Sodium Electrode

3. Magnetic stirrer
4. Glassware and plastic ware

Reagents and standards

1. Distilled water
2. Sodium Ionic Strength Adjustment Buffer (ISA) - 4M NH_4C1 and 4M NH_4OH.
3. Sodium Standard Solution. (270.6 g $NaNO_3$ diluted to 1000 mL - 100,000 ppm)

Procedure

Sample Treatment

1. 50 ml of the sample was transferred in to a plastic beaker and the sample temperature was ensured to be the same as that of the standards used in calibration.
2. The stirring bar was Added and mixed on the magnetic stirrer. It was mixed gently to avoid air bubbles from drawn into the solution.
3. The level of inner filling solution was checked and filled.
4. The electrode was immersed into the sample making sure sample volume was above collar of electrode.
5. Sodium ionic strength adjustment buffer was added, so that the ratio of buffer to sample was (1:10) (i.e., for 50 ml of sample, 5 ml buffer was added).
6. The pH of the sample was checked with pH meter and it was greater than 6.
7. When the electrode come to equilibrium, then the electrode potential of the sodium concentration in mill volts was measured.

Sample Measurement

8. The observed potential in mill volts was recorded and converted to milligrams per liter of sodium by means of the calibration curve then results were recorded.

Figure3.1 shows some equipments used during the study AD1020 pH/mv/ISE and T meter for temperature and pH and Spectrophotometer MD600 for the anions and cations concentration measurement.

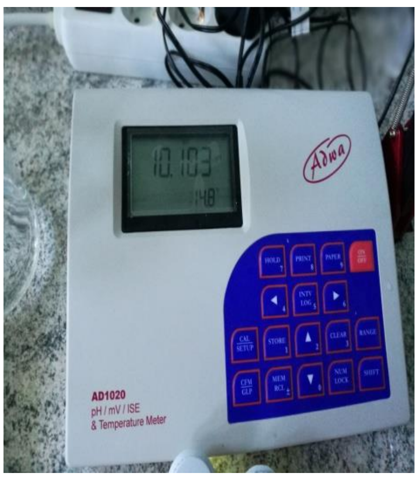

Figure.3.1. a. pH/mv/ISE and T meter AD1020

Figure.3.1. b. Spectrophotometer MD600

Figure.3.1. c. AD300 EC/TDS meter

Figure.3.1. d. Colony Counter

Figure 3.1. Some equipment used during the study

The heavy metals Analysis

Flame Atomic Absorption Spectroscope Apparatus (FAAS)

For the analysis of the heavy metals Cr, and Cd, FAAS, BUCK SCIENTIFIC MODEL 210VGP atomic Absorption Spectroscopy equipped with deuterium ark background correctors, cathode lamps for each respective element, and air acetylene flame were used.

3.5.2 Bacteriological Analysis

The bacteriological parameters considered in this study were total coli forms, fecal coli forms, fecal streptococci and heterotrophic plate count (HPC). Sample analyses were done according to the international standards for the examination of water ISO 9308-1, 2000.

Enumeration of total coli forms, fecal coli forms and fecal streptococci

Total coli forms, fecal coli forms and fecal streptococci were determined using membrane filtration (MF) method developed to assess the degree of contamination in water , WHO, 2006; ISO 9308-1, 2000.

One hundred milliliter of water sample for each test was filtered through a sterile cellulose membrane filter with a pore size of 0.45μm to retain the indicator bacteria. The filtration apparatus was sterilized before use and re-sterilized between samples using methanol when analyzing water samples. Each time after filtration, the cellulose membrane filter was transferred from filtration apparatus to a sterilized aluminum petri-dish containing absorbent pad soaked with membrane lauryl sulphate tryptose broth for total coli forms (TC) and fecal coli form (FC); and M. enterococci agar for fecal streptococci (FS) were used.

The media and bacteriological loaded Petri dishes were then incubated at 30ºC and four hours for TC and 37ºC for 4 hours for both FC and FS, to permit bacterial growth. The Petri-dishes were inverted and incubated at 37ºC for 18-24 hours for TC and 44ºC for 18-24 hours for FC and FS.

Determination of the heterotrophic plate count (HPC)

Heterotrophic plate count (HPC) is a procedure used for estimating the number of live heterotrophic bacteria in water that form colony on culture media. The heterotrophic bacteria were determined by the membrane filtration methods using Hetro plate Count Agar (HPCA) media. The media was prepared according to the standard methods from the basic ingredients such as peptone (139 gm), soluble casein (124gm). K_2HPO_4 (92gm), $MgSO_4$ (86gm), $FeCl_2$ (70gm), agar (205gm) and reagent graded water. The pH of the media was adjusted to 7.2 with 1.0N NaOH and was autoclaved for 15 minutes at 121^0C. About 10ml of sterile medium were dispensed into petri dishes. All the filtering apparatus were sterilized before filtration and 100ml volume of water was filtered through 0.45µm pore size gridded membrane filter. After filtration, the petri dishes were incubated at 28^0C about 5 days. And colonies were counted and recorded.

Chapter 4: Results and Discussion

4.1 Physical and chemical Analysis

4.1.1 Classification of bottled Water brands

Different hydro-chemical classification systems are available to classify water types. In the present case three directives or standards were used for the classification and comparison purpose.

- The European Union (EU) mineral water directive: this was used to classify the investigated natural mineral waters based on their TDS level .This standard was used due to the Ethiopian natural mineral water standard lacks such classification.
- The Ethiopian bottled waters standard (other than natural mineral waters) CES99 : based on this standard ,the bottled waters were classified by evaluating the obtained TDS and TH values and;
- The Ethiopian Natural Mineral Water Standard (CES151): This standard was also used to classify the bottled waters based on their TDS.

After classification of the bottled waters using the above standards, they were also compared with other countries bottled waters parameters. The classification used was to identify the chemical similarities and/or differences among the water brands. In addition the Ethiopian standards were also compared with the other international standards. The EU mineral water directives for the criteria of chemical composition were presented in the Table 4.1. From this table, it is understandable that the criteria was formulated based on TDS accompanying with the concentration levels of cations and anions. Table 4.3

shows the classification of presently observed data for bottled water arranged according to the EU mineral water directive. It can be seen from table 4.1 that the coded brands B, C and F were falling in the "very low mineral concentration" class as the TDS value found to be 9, 33 and 10 mg/L respectively. The water brands coded A, D and E were in the "low mineral concentration" class. On the other hand, coded brand B, C, D, E and F are falling into the soft water category as the observed TH values are within the concentration range of 0-50 mg/L. The brand A is considered to be moderately hard water .The classification of the bottled waters was seen in table 4.2.

Table 4.1. Classification of water based on EU directive [88]

Water type	Criteria
Very low mineral concentration	Mineral content (TDS) < 50 mg/l
Low Mineral concentration	TDS <150mg/l
Intermediate mineral concentration	TDS 500-1500 mg/l
High mineral concentration	TDS > 1500 mg/l
Containing sulphate	Sulphate > 200 mg/l
Containing chloride	Chloride > 200 mg/l
Containing calcium	Calcium > 150 mg/l
Containing magnesium	Magnesium > 50 mg/l
Containing iron	Bivalent iron > 1 mg/l

Table 4. 2. Classification of drinking water based on TH in mg/l [88].

Classification	$CaCO_3$ equivalent (mg/L)
Soft	<75
Moderately hard	75–150
Hard	150–300
Very hard	>300

Table 4.3. Classification of drinking water based on TDS and TH in mg/L (Present study)

Brand code	TDS	EU Class	TH	Water class
A	190	Low mineral concentration	120	Moderately hard
B	9	very low mineral concentration	3	Soft
C	33	very Low mineral concentration	20	soft
D	82	Low mineral concentration	20	Soft
E	140	low mineral concentration	34	Soft
F	10	very Low mineral concentration	26	Soft

4.1.2 Physical and Chemical properties study

pH

It was mentioned earlier that the pH of the water samples were determined by ADWA 1020 pH meter. The observed pH values including other data's were placed in Table 4.4; and it may be seen that the pH values ranges in between 5.9-7.2, with a median value of 6.95. It shows that 50% of the water samples were slightly acidic. Table 4.5 represents the values of different parameters of drinking water of CES and WHO. From the Tables 4.4 and 4.5, it may be seen that only the water of the coded brand B stands out of the recommended pH value of CES and WHO; whereas water of the coded brands A, C, D, E and F stands within the range of the recommended values, i.e., accepted values but there is significant difference among the brands.

Table 4.4: Concentration of major constituent of bottled drinking water brand (Present Study)

Parameter	Brand Code (mean values)						Mean	Min.	Max.	SD	Median
	A	B	C	D	E	F					
pH	7.2	5.9	6.9	7	7.2	6.6	6.8	5.9	7.2	0.49	6.95
EC, μS/cm	380	18	66	164	280	20	154.7	18	380	149.16	115
TDS, mg/L	190	9	33	82	140	10	77.3	9	190	74.58	57.5
TH, mg/L	120	3	20	20	34	26	37.2	3	120	41.84	23
Cl-, mg/L	12	1.1	2	7.4	6.4	1.4	5.05	1.1	12	4.33	4.2
NO3-,mg/L	1.8	0.1	2	5	5	3	2.82	0.1	5	1.93	2.5
SO4, mg/L	0	0.5	8	7	9	1.2	4.28	0	12	4.14	4.1
PO_4^{3-} mg/L	ND	ND	ND	ND	ND	ND	-	-	-	-	-
F^- mg/L	0.26	0.2	0.31	0.1	0.15	0.1	0.19	0.1	0.26	0.09	0.175
Na^+ mg/L	15	3	9.5	5	10	1	7.25	1	15	5.19	7.25
K^+ mg/L	4.7	0.25	0.5	1	0.68	0.7	1.31	0.25	4.7	1.68	0.69
Ca^{2+} mg/L	35.27	0.23	10	10	20	1.2	12.78	0.23	35.27	13.15	10
Mg^{2+} mg/L	7.8	0.21	1.95	2.5	3	1.2	2.78	0.21	7.8	2.65	2.225
Fe(total) mg/L	0.1	0.1	ND	ND	0.2	0.1	-	0.1	0.1	0.05	0.1
Cr^{3+}	ND	ND	ND	ND	ND	ND	-	ND	ND	-	-
Cd^{2+}	ND	ND	ND	ND	ND	ND	-	ND	ND	-	-
Zn^{2+}	0.1	0.02	0.02	0.05	0.02	0.02	0.04	0.02	0.1	0.03	0.02
Color TCU	3	1	1	2	2	3	2	1	3	0.89	2
Turbidity	1.5	ND	ND	1	1	1	-	ND	1.5	0.25	1

EC and TDS

From the Table 4.4, it may be seen that the EC values of the bottled waters are appeared to be in the range of 18–380μS/cm. The gap in between the lowest and the highest EC values is wide enough and the median is 115μS/cm. It is recognized that EC is usually the measure of ionic concentrations present in a water sample. Therefore, it can be speculated that the sample coded B,C,D,E and F consists of low ionic concentrations and hence lies in the bottled water range whereas the sample coded A contains high ionic concentrations and lies in the Ethiopian Natural mineral water standard range . Such a variation of ionic concentration may appear from the surrounding environment of water source and treatment system used. On looking the obtained TDS values, it may be seen that the values are appeared in the range of 9-190 mg/L with the median 57.7 mg/L.

The remarkable fact that the lowest EC valued sample B showed the lowest TDS value and the highest EC valued sample A showed the highest TDS value. Such coincidences among the EC and TDS values may be taken as the accuracy of the measurements. The color and turbidity of all the bottled waters analyzed are within the standard limit.

Total Hardness (TH)

Total hardness of water mainly represents the concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, in the form of carbonate and bicarbonate. From the Table 4.4, it may be observed that the total hardness values of the bottled waters are found from 3 – 120 mg/L. The difference between the highest and lowest value is so significant and the median is 23. Among the samples, it was observed that brand A water sample contained highest amount of TH and the lowest amount was found in B and F coded sample. Water can be classified as soft (<75 mg/L), moderately hard (75-150 mg/L), hard (150-300 mg/L) and very hard (>300 mg/L) according to the concentration of calcium and magnesium as table 4.2. It is an important criterion for determining the usability of water for domestic, drinking and many industrial applications. Water having hardness below 300mg/L is considered potable, but beyond this limits cause gastro-intestinal irritation and taste problems for bottled water. But it is very important to limit the lower value of the hardness as it directly related to calcium and magnesium levels and this is the gap seen in the Ethiopian bottled water standard (CES99) in comparison to the WHO standard.

Concentration of ionic constituents

The concentration level of the ionic constituents present in the water samples are: 1.2 - 35.27 mg/L for Ca^{2+} with a median of 10 ; 0.21-7.8 mg/L for Mg^{2+} with a median of 2.23; 0.1-0.20 mg/L for Fe^{2+} with a median of 0.1 ; 1.1 -12 mg/L for Cl^{-} with a median of 4.2 ; 0.0-9.0 mg/L for SO_4^{2-} with a median of 4.1; and 0.1-5 mg/L for NO_3^{-} with a median of 2.5 (Table 4.4). Both calcium and magnesium are essential to human health. Inadequate intake of either nutrient can impair health which has been associated with increased risks of osteoporosis, colorectal cancer, hypertension and stroke, coronary artery disease, insulin resistance and obesity. On top of this the water brands A, B, E, F label them themselves as natural mineral water without containing minerals.

Table 4.5. Physico-chemical parameters of drinking water according to the CES and WHO

Parameter	Unit	CES 99 (for purified bottled water)	CES 151 (for bottled natural mineral water)	WHO
pH	-	6.0 – 8.5	6.0-8.5	6.5 – 8.5
EC	µS/cm	-	-	-
TDS	mg/L	1000	150-1500	1000
TH	mg/L	300	400	80 - 120
Cl^{-}	mg/L	250	200	400
NO_3^{-}	mg/L	50	50	50
SO_4^{2-}	mg/L	250	200	500
Fe^{2+}	mg/L	0.3	0.3	0.3
Ca^{2+}	mg/L	75	200	200
Mg^{2+}	mg/L	50	100	50

On the other hand, excessive intake of these constituent minerals may be a cause for different diseases like kidney stone, osmotic diarrhea etc. From Table 4.4, it can be seen that sample coded A has higher concentration of Ca^{2+} and Mg^{2+} as compared to the others. It may be also found that Ca^{2+} concentration was higher than Mg^{2+}, among the brands; this means that hardness of water comes mostly from Ca^{2+} concentration. Fe^{2+}concentration were found at high level in water brand F actually less than the maximum limit which is 0.2mg/l. This may be occurred due to methods of processing and source of water that ensure the higher concentration of Fe^{2+} or corrosion of metal surfaces if used for piping instead of stainless steel. A report has been shown that uptake of iron supplements for extended periods without deleterious effects and an intake of 0.4–1 mg/kg of body weight per day is unlikely to cause adverse effects in healthy persons [2]. Cl^- and SO_4^{2-} are not of health concern at levels found in drinking-water, however, excessive Cl^- concentrations increase rates of corrosion of metals in the distribution system and when it exceeds over 600 mg/L, impair the portability of water. SO_4^{2-} is one of the least toxic anions. The presence of high concentration of SO_4^{2-}in the drinking water may lead to dehydration, stomach complaints, and possibly diarrhea. In general, the adverse effect on the taste is said to be minimal at levels lower than 250 mg/L for both Cl^- and SO_4^{2-}. NO_3^- in drinking water can also affect certain adults and small children. It was observed that sample coded D and E contains higher concentration (5 mg/L) of NO_3^- compared with the other samples, which is within the minimum range of WHO and CES limits (Table 4.5). Pregnant women can pass methemoglobin on to developing fetuses and low birth weights have been attributed to high nitrates in water. The observed variations in the chemical constituents can be described as the variation of the origins, residence time, atmospheric conditions and purification or treatment process employed by the manufacturers. In fact, it may be showed that the concentration of the ionic constituent present in the collected bottled water samples are within the recommended drinking water CES99 and WHO (Table 4.5) standard limits except for those bottled waters who label Natural mineral water all fail in comparison to this standard except brand A. This is because a bottled to be natural mineral water the TDS must be in between 150-1500mg/l as per CES 151.

Contribution of ions

It was found that sample coded A contains exceptionally high levels of most ions like Cl^-, Ca^{2+} ,Mg^{2+} ,Na^+ and K^+ compared with the other water samples. The contributions of the measured ions as it may be seen that Ca^{2+} was the dominating component in the A, C, D and E coded water samples whereas $SO_4^{2-}$was dominated in the water samples coded C, D and E. A particular fraction of Fe^{2+} was observed, in the range of higher level in brand E. Somewhat higher proportion was found for Mg^{2+} (7.8 mg/l) in brand A. It may be think that the source of SO_4^{2-}comes from the oxidation of sulfate containing ores like gypsum and Cl^- may be arose from water that entrapped in the sediments during chlorination. The contribution of Fe^{2+} in water is common in deeper wells where the water has been in contact with rock for a longer time.

Correlation between various constituents

Table 4.6 shows the Pearson's correlation between physicochemical characteristics of water samples and the metals ion (Ca^{2+}, Mg^{2+} and Fe^{2+} etc.) concentration. According to Taylor, the correlation coefficient, $r \leq 0.35$ represent weak correlations, r value of 0.36-0.67 indicate moderate links, and r value of 0.68-1.00 signify strong relationships. Bottled water samples show strong positive correlation (r= 0.68-1.00) for pH and TH, between EC and TDS, TH , $Cl^{-,}$ Ca^{2+}, and Mg^{2+}, between TDS and TH, Cl^{-}, Ca^{2+} and Mg^{2+}, between TH and Cl^{-}, SO_4^{2-}, Ca^{2+}, and Mg^{2+}, between Cl^{-} and Ca^{2+}, and Mg^{2+}, and also between Ca^{2+}, and Mg^{2+},while moderate correlation (r=0.36-0.67) exit between K^{+-} and PH, F^{-} ,between F^{-} ,Ca^{2+} , and Mg^{2+} and weak correlation ($r \leq 0.35$) exits between pH and Fe^{2+},between EC and NO_3^{-}, between TDS and NO_3^{-}, Fe^{2+}, between TH and NO_3^{-} and also between SO_4^{2-} and Fe^{2+} .

Table 4.6. Correlation matrix for water quality parameters in the bottled drinking water brands

	PH	**EC**	**TDS**	**TH**	**Cl^{-}**	**NO_3^{-}**	**$SO_4,^{2-}$**	**F^{-}**	**Na^{+}**	K^{+}	Ca^{2+}	Mg^{2+}	Fe
pH	1												
EC	0.71	1											
TDS	0.71	0.99	1										
TH	0.57	0.9	0.9	1									
Cl^{-}	0.7	0.95	0.95	0.83	1								
NO_3^{-}	0.6	0.1	0.1	-0.1	0.3	1							
SO_4^{2-}	-0.2	-0.06	-0.06	0.96	-0.02	-0.68	1						
F^{-}	0.07	0.23	0.23	0.33	0.07	-0.59	-0.047	1					
Na^{+}	0.7	0.86	0.86	0.78	0.75	-0.21	0.16	0.6	1				
K^{+}	0.4	0.8	0.8	0.8	0.84	-0.1	-0.44	0.33	0.68	1			
Ca^{2+}	0.75	0.98	0.98	0.9	0.9	0.6	0.01	0.3	0.93	0.86	1		
Mg^{2+}	0.75	0.96	0.96	0.97	0.92	0.08	-0.17	0.33	0.85	0.95	0.96	1	
Fe	0.05	0.3	0.3	0.1	0.12	0.11	-0.11	-0.11	-0.2	0.12	0.2	0.1	1

Heavy Metals Analysis

Analytical Procedures for Heavy Metal Analysis

In this study, the concentrations of the heavy metals chromium and cadmium in all popular commercially available bottled water available in Addis Ababa (A, B, C D, E, and F) samples were quantitatively determined using FAAS. Atomic absorption spectroscopic standard solutions containing 1000 mg/L were used for preparing intermediate standard solutions (10 mg/L) in 100 mL volumetric flask and working standards using deionized water. Working standards of metal solutions were prepared in 50 ml volumetric flask by diluting with deionized water and the data are given in Table 4.7. Four points of calibration were established by running the prepared standard solutions in flame atomic absorption spectrometer. Immediately after calibration, the sample solutions were aspirated into the FAAS instrument and direct readings of the metal concentrations were recorded. Three replicate determinations were carried out on each sample. The operating conditions of FAAS are given in Table 4.8.

Table 4.7. Concentration of standard solutions for FAAS instrument calibration and correlation coefficient of calibration curves.

M	Concentration of the standards in (mg/L)	Correlation coefficient
Cr	0.1, 0.2, 0.4, 0.8	0.9990
Cd	0.1, 0.2, 0.4, 0.8	0.9999

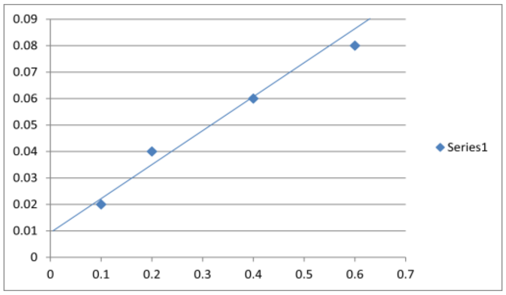

Figure 4.1. Calibration curve for Cr

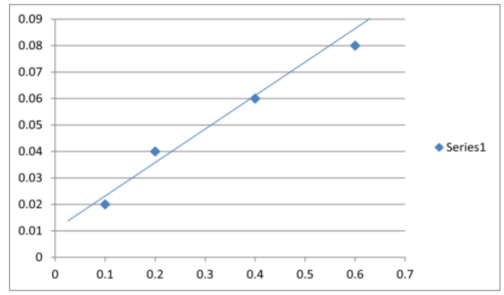

Figure 4.2. Calibration curve for Cd

Table 4.8. FAAS Instrument operating conditions (test parameters)

Element	Wavelength (nm)	Detection limit (mg/L)	Slit width (nm)	Current (mA)	Energy (EV)
Cr	357.9	0.05	0.7	2	3.759
Cd	228.9	0.005	0.7	2	3.338

Determination Method and Detection Limits

The detection limit was taken the minimum concentration of analyte that was identified, measured and reported with 99% confidence as the analyte concentration was greater than Zero.

The detection limit was accepted and taken, as the concentration gave a signal three times the standard deviation of the blank or background signal [89].

In this study the method used for the detection limit for the analysis of heavy metals using FAAS in water samples were determined using reagent blank, i.e. 1 ml of 69-72% HNO_3, which was used for acidifying the sample solutions, and was added to 50 ml of deionized water that was used for washing apparatus and for the dilution of standard solutions. The instrument detection limit was taken for the determination of each metal.

The water sample was directly taken from the bottles, injected to the column and analyzed.

Table 4.9. Instrument method detection limits for the analysis of water samples by FAAS.

Detection limits		
Element	IDL (mg/L)	MDL (mg/L)
Cr	0.05	0.05
Cd	0.005	0.005

Determination

The two heavy metals (Cr, and Cd) were determined using flame atomic absorption spectroscopy (FAAS) method as recommended by United State Environmental Protection agency [94]. The results shows that Chromium and cadmium were below the detection limits in all samples and are reported as not detected (ND).

Cadmium: Cadmium was found to be below instrument detection limit in all the water samples, which is within the WHO guideline for drinking water 0.003 mg/L.

Chromium: The chromium concentration was also found to be below the instrument detection limit as mentioned above in all water samples, hence this undetected value shows the level of chromium in drinking water is not exceeded the current guideline value of 0.05 mg/L [1].

Comparison of bottled water samples with their labeled parameters

The current results were compared with that of labeled parameters on the label of the bottles using t- test statistically as shown in Table 4.10. T-test is usually used to compare weather there is any significant difference between the true mean and the measured mean values of a given analytical data. The greater the magnitude of t (it can be either positive or negative), the greater the evidence against the null hypothesis that there is no significant difference. In this study in brand A and C there is significant difference as the t calculated is greater than the t critical which is 0.05.In the other brands the t calculated is greater than the t critical and hence the difference is insignificant among the labeled and actual parameters. This is because the bottled waters labeled B, D, E and F, as can be seen from the analysis have very low minerals content or are de-mineralized table 4.10.

Table 4.10. Comparison of analytes on the labels with current results in mg/L

ANALYTE			ANALYZED MEAN VALUES OF THE SAMPLES															
	A			B			C			D			E			F		
	Label	current	t-value	Label	current	t-value	Label	current	t-value	Label	current	t-value	Label	current	t-value	Label	current	t-value
Cl	24	12	0.050	5.53	1.1	0.126	1.1	2	0.034	7.6	7.4	0.104	6.38	6.4	0.326	1.4	1.4	0.383
Na^+	19.1	15		2.7	3		3	9.5		1.08	5		8.48	10		1.2	1	
K^+	7.7	4.7		0.28	0.25		0.5	0.5		1.65	1		0.68	0.68		0.7	0.7	
Ca^{2+}	54.8	35.27		0.21	0.23		1.1	10		3.2	10		15.35	20		1.2	1.2	
Mg^{2+}	15.7	7.8		0.14	0.21		0.5	1.95		1.95	2.5		1.92	3		0.8	1.2	
PH	7.5	7.2		7	5.9		7.2	6.9		7	7		7.24	7.2		7	6.6	
TDS	250	190		30	9		20	33		82	82		144	140		10	10	

Table 4.11. Comparison of Labeled name versus to –be as per the standard requirement

ANALYSED BRANDS											
A		**B**		**C**		**D**		**E**		**F**	
Labeled name	Standard name	Labeled name	Standard name	Labeled name	Standard name	Labeled name	Standard name	Labeled name	Standard name	Labeled name	Standard name
Natural Mineral Water	Natural Mineral Water	Natural Mineral Water	Purified/RO/distilled water	natural purified water	Natural Purified water	Spring Water	Purified water	Natural Mineral water	Purified /RO /Distilled water /	Natural mineral	Purified /RO/Distilled Water

As can be seen from table 4.11 about 67% of the bottlers wrote a misleading label as compared with the standard requirement. Regarding the minerals content; our bottled waters are in a very low range almost with no natural minerals that can affect health (table 4.12). When our sample bottled waters are compared with the national and international standard; the minerals content is in a very low range except in brand A.

Table 4.12. Comparison of current results with some national & international guidelines

Guidelines		Average concentrations (mg/l)							
		Cl^-	F^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
WHO		250	1.5	50	250	200	-	50	200
EC		250	1.5	50	250	200	-	-	-
USEPA (1993)		250	2	44	250	-	-	-	-
Ethiopian	CES 99	250	1	50	200	200	50	50	75
	CES151	200	1	50	200	300	50	100	200
Brand A		12	0.26	1.8	200	15	4.7	7.8	35.27
Brand B		1.1	0.2	0.1	0.5	3	0.25	0.21	0.23
Brand C		2	0.31	2	8	9.5	0.5	1.95	10
Brand D		7.4	0.1	5	7	5	1	2.5	10
Brand E		6.4	0.15	5	9	10	0.68	3	20
Brand F		1.4	0.1	3	1.2	1	0.7	1.2	1.2

Table 4.13. Comparison of the results obtained for both bottled and mineral waters with other countries similar waters [90].

Country	**Analytes average values for both water types**					
	F^-	Cl^-	Na^+	Mg^{2+}	K^+	Ca^{2+}
Ethiopia	0.1-0.31	1.1-12	1-15	0.21-7.8	0.25-4.7	0.23-35.27
Egypt	0.12-0.48	11.1-221.1	4.94-169	1.54-23.3	0.11-18.5	1.39-44.8
Australia	0.10-0.22	5.9-47.4	2.4-34.5	5.7-38.6	0.7-20.0	0.5-4.6
Portugal	0.0-0.05	8.6-15.8	7.6-11.8	1.5-13.6	1.5-13.6	0.0-22.1
Canada	0.2-0.36	0.0	0.0-1.5	0.2-6.0	0.2-6.0	3.0-7.9
China	0.07-0.79	0.0-67.0	8.1-31.4	0.4-24.1	0.4-24.1	0.7-1.4

We can see from table 4.13, that there are a lot of variations of the Ethiopian bottled water samples with other countries sample waters.

4.2 Microbiological Analysis

Occurrence of Total coli forms, faecal coli forms and faecal streptococci in the bottled water samples

The microbiological analysis of the different bottled water samples are shown in table 4.8. The samples show slight difference amongst one another. From a total of 18 bottled water samples taken directly from the supermarkets one bottle sample from brand B and two bottle samples from brand C were found to be positive for total coli forms (TC) from the bottle samples produced at different times .This may show that there may not be product quality and safety consistency during production and treatment process as contamination after disinfection and capping is rare. None of the samples from brand A, D, E and F was positive for TC. The presence of TC in water sample may indicate the ineffectiveness of the treatment systems of the two brands before bottling. In the present study, no fecal coli forms and fecal streptococci were detected from all samples.

Table 4.14. Occurrence of indicator bacteria from water samples of the six brands

Brand	TC	FC	FS
A	0(0%)	0(0%)	0(0%)
B	1(5.5%)	0(0%)	0(0%)
C	2(11.1%)	0(0%)	0(0%)
D	0(0%)	0(0%)	0(0%)
E	0(0%)	0(0%)	0(0%)
F	0(0%)	0(0%)	0(0%)

Key: - TC (Total Coli forms), FC (Fecal Coli forms), FS (Fecal streptococci)

In this study, brand B and C were not found to be suitable for human consumption since they failed to meet the World Health Organization and compulsory Ethiopian standards in which total coli forms should not be detected in bottled drinking water. Even though the number of positive samples was low, the presence of coli forms in bottled water suggests the potential presence of pathogenic enteric microorganisms such as *Vibrio cholera*, *Salmonella* spp, which were not studied in this sample and that their presence in bottled drinking water might pose a health risk to consumers. Gastroenteritis epidemics caused by this pathogen following consumption of contaminated bottled drinking water have been reported.

The CES99 and CES151 -2015 and WHO, 2004a sets zero detection per 100 ml for total coli form bacteria. The presence of fecal coli forms in bottled water shows that there is a contamination from either human or animal excreta. It may be from ineffective treatment sytem, production process and /or hygiene and sanitation system or by cross contamination of unhygienic environment.

Table 4.15. Distribution of over all Heterotrophic plate count in bottled water in the six brands

Brands	Number sample examined	HPC count (CFU/ml) in percent		
		0-50	1-500	>500
A	3	100%	0%	0%
B	3	0%	0 %	100%
C	3	0%	0%	100%
D	3	26.6%	73.4%	0%
E	3	100%	0%	0%
F	3	66.7%	33.3%	0%

The heterotrophic bacteria counts from the bottled water samples showed that most of the bottled-water brands were contaminated as shown in table 4.15. All 18 samples of brands A, B, C, D, E and F tested positive for the presence of heterotrophic bacteria .Water brands B and C, 100% samples examined indicated the presence of heterotrophic organisms more than 500 CFU/ml which fails to meet WHO drinking water quality specifications which allow HPCs of as high as 500CFU/ml. For brand A, 100% of the samples tested were between 0-50 and D 26.6 % was between 0-50 and the rest 73.4 % was 1-500. The lowest percentage was recorded in brand A in which all samples examined were less than 50 CFU/ml.

There is no evidence that HPC values alone directly relate to health risk either from epidemiological studies or from correlation with occurrence of waterborne pathogens. However, specific strains of microbial species that may be part of HPC micro biota can cause infection in certain vulnerable people especially the immuno-compromised [91]. These differences within the same brands but collected from different sites and different production dates may be due to the cross contamination during handling, transportation and storage, ineffective treatment system and unhygienic working condition, both in the producers and retailers. As the results of the study show, there was also a very high significant difference between the six brands investigated in this study with regard to chemical composition.

Chapter 5: Conclusion and Recommendation

5.1 Conclusion

Water is essential for life and its increasing consumption in bottled form for drinking as well as the necessity to protect and inform the consumers explains the interest to study its quality and safety. As a result, in this study six brands of bottled waters sold in Addis Ababa City, Ethiopia, were assessed for the physical, chemical and microbial parameters.

Based on the results of this study, there is a large variation in composition and naming among the brands and with the standard requirement compared, thus the consumer should take due care while choosing the bottled waters for consumption. The inorganic composition of a number of bottled water brands samples from different locations were determined and compared with the standard requirement and among themselves. The common cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and TDS concentrations are higher in natural mineral water brand A which fulfils the requirement as per the CES and WHO guide lines but in the other brands the concentrations are far below the requirement and most of them are not recommended for drinking as per WHO standards .Heavy metals were not detected in all the six brand water samples. The anion concentrations are also varying among the brand samples.

There is a contradiction in naming the bottled waters that can mislead consumers. Two brands among the three natural mineral waters used and labeled wrong names and declare importance of minerals while they don’t contain them in the desired level. Only brand A fulfills the mineral water requirement and labeled accordingly.

The Ethiopian standard doesn't have the minimum required TDS value for bottled waters. These allow bottled waters producers to produce Demineralized water that can affect consumer's health in long term consumption.

It is advisable for people who have a problem of blood pressure, kidney, heart disease and circulatory illness to drink lower TDS value waters; though it is difficult to rely on the label as there is arbitrary naming.

The results obtained in this research were compared with some of the national and international guidelines. All the parameters determined were far below the guideline limits. It is advisable to consume bottled waters with pH values above 7 but most brands pH is lower and that of brand B is out of the CES and WHO standard requirement.

The importance of the quality of water for human consumption with regard to health makes it necessary to establish norms to regulate it, including limits for all the parameters that directly affect human health and deteriorate water quality.

The results of this study were also compared with the results of other countries' mineral water in the world. Except some outlined results reported from different countries, the composition of the bottled waters in Ethiopia is more or less similar to that of other countries, though no data was found whether they are bottled or natural mineral water.

During the visit it was observed that all of the bottling water companies except brand A, use extreme filtration of Reverse Osmosis /demineralization/. Regardless of the source, wells or springs when they use extreme filtration it is proven over and over that they create dead-water. Dead water is void of all healthy minerals, similar to acid rain. If this water is to come to the standard re-mineralization or fortification must be used.

Brand A bottler uses ultra-filtration system to keep the healthier minerals in the water for health purpose. Using RO makes the water acidic that is why most of them are in the acidic range.

Regarding to microbial parameters, brand B and C were not found to be suitable for human consumption since they failed to meet the WHO and CES in which total coli forms was identified. Brands B and C, was found 100% of their samples examined indicated the presence of heterotrophic organisms more than 500 CFU/ml .This makes it fail to meet WHO drinking water quality specifications which allow HPCs of as high as 500CFU/ml . For brands A 100% of the samples tested was between 0-50 and D, 26.6 % was between 0-50 and the rest 73.4 % was 1-500. The lowest percentage was recorded in brand A in which all samples examined were less than 50 CFU/ml.

Generally, bottled water brands are projects of huge sum of money, which got that way by convincing consumers that their bottled water is cleaner and healthier than tap water. However the comparisons of bottled water label and actual have shown that there is a significant difference and the regulatory body is relaxed. The importance of the quality of water for human consumption with regard to health makes it necessary to establish norms to regulate it, including lower and upper limits for all the parameters that directly affect human health and deteriorate water quality.

5.2 RECOMMENDATION

The study covers a limited number of branded bottled waters, it is recommend that all marketed bottled waters be monitored for treatment methods used, quality, safety, hygienic and facility requirements and be licensed by the concerned authorities to safe guard consumers‘health and ensure the sustainability.

Finally, I recommend further studies should focus on:

- The possibility to verify the Ethiopian bottled waters taking into account of increased number of samples and additional parameters like heavy metals lead, mercury, arsenic and pesticides and THM’s.
- Shelf life analysis of bottled waters and validation of the bottled water at different environmental conditions
- Effects of consumption of de-mineralized water to human health
- Suitability of the compulsory Ethiopian Standard to produced and supply safe and quality product

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Appendices

Appendix A1: Determination of pH

Procedure:

1. Press RANGE to enter pH range. Press RANGE again to change the pH measurement resolution.
2. Immerse pH electrode tip and temperature probe approximately 4 cm into the solution to be tested. Allow for the electrode to stabilize and put the temperature probe tip as close as possible to the pH electrode.
3. The pH reading is displayed on the primary LCD and the temperature value on the secondary LCD.
4. The pH reading is affected by temperature. In order to measure the pH accurately, the temperature effect must be compensated for. To use the ATC (Automatic Temperature Compensation) capability of the instrument, connect the AD7662 temperature probe, immerse it into the sample as close as possible to the pH electrode and wait for a few seconds.

Appendix A2: Determination of EC and TDS

SANSEL digital TDS meter was used to measure the TDS and EC of the sample waters by simply:

1. Press the on/off switch
2. Manually select the estimated range of the sample
3. Adjust and fixed the temperature to 25^{o}C
4. Rinsing the probe in distilled water

5. Dipping the electrode in the sample water and stirring gently
6. Wait until the reading stops flashing, till the reading becomes stable
7. Use the MODE key to switch from TDS to Conductivity and vice versa or divide conductivity by 2 to get TDS

Appendix A3: Determination of Turbidity:

Procedure:

1. Fill a clean vial (24 mm Ø) with 10 ml of de-ionized water, close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber and empty the vial completely.
5. Stir the water sample. Immediately rinse the vial with the water sample and fill with 10 ml water sample.
6. Close the vial tightly with the cap and swirl gently several times.
7. Place the vial in the sample chamber making sure that the marks are aligned.
8. Press TEST key and the result is shown in the display in FAU.

Appendix A4: Determination of Color (true and apparent):

Procedure:

0 – 500 Pt-Co units

Sample preparation):

Step A

- Filter approx. 50 ml deionised water through a membrane
- Filter with a pore width of 0.45 µm.

- ➢ Discard the filtrate. Filter another 50 ml deionised water and keep it for zeroing.

Step B

Filter approx. 50 ml water sample using the same filter.

Keep this filtrate for sample measurement.

1. Fill a clean vial (24 mm Ø) with 10 ml of the filtrated deionised water (from Step A), close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber and empty it completely.
5. Rinse the vial with the filtrated water sample and fill with 10 ml filtrated water sample (from Step B).
6. Place the vial in the sample chamber making sure that the marks are aligned.
7. Press TEST key.

The result is shown in the display in Pt-Co units.

Appendix A5: Determination of Chloride (Cl^-):

Procedure

0.5 – 25 mg/l Cl

1. Fill a clean vial (24 mm Ø) with 10 ml of the water sample, close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber.
5. Add one CHLORIDE T1 tablet straight from the foil to the water sample, crush the tablet using a clean stirring rod and dissolve the tablet.

6. Add one CHLORIDE T2 tablet straight from the foil to the same water sample and crush the tablet using a clean stirring rod.
7. Close the vial tightly with the cap and swirl gently several times until the tablet is dissolved (Note 1).
8. Place the vial in the sample chamber making sure that the marks are aligned.
9. Press TEST key. Wait for a reaction period of 2 minutes. After the reaction period is finished the measurement starts automatically. The result is shown in the display in mg/l Chloride.

Appendix A6: Determination of Total Hardness:

Hardness, total with Tablet 2 – 50 mg/l CaCO3

1. Fill a clean vial (24 mm Ø) with 10 ml of the water sample, close tightly with the cap.

2. Place the vial in the sample chamber making sure that the marks are aligned.

3. Press ZERO key.

4. Remove the vial from the sample chamber.

5. Add one HARDCHECK P tablet straight from the foil to the water sample and crush the tablet using a clean stirring rod.

6. Close the vial tightly with the cap and swirl several times until the tablet is dissolved.

7. Place the vial in the sample chamber making sure that the marks are aligned.

8. Press TEST key.

Wait for a reaction period of 5 minutes. After the reaction period is finished the measurement starts automatically. The result is shown in the display as total Hardness.

20 – 500 mg/l CaCO3

1. Fill a clean vial (24 mm Ø) with 1 ml of the water sample and 9 ml of de-ionized water, close tightly with the cap.

2. Place the vial in the sample chamber making sure that the marks are aligned.

3. Press ZERO key.

4. Remove the vial from the sample chamber.

5. Add one HARDCHECK P tablet straight from the foil to the water sample and crush the tablet using a clean stirring rod.

6. Close the vial tightly with the cap and swirl several times until the tablet is dissolved.

7. Place the vial in the sample chamber making sure that the marks are aligned.

8. Press TEST key.

Wait for a reaction period of 5 minutes. After the reaction period is finished the measurement starts automatically. The result is shown in the display as total Hardness.

Appendix A7: Determination of SO_4^{-2}

5 – 100 mg/l SO4

1. Fill a clean vial (24 mm Ø) with 10 ml of the water sample, close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber.
5. Add the contents of one VARIO Sulpha 4/ F10 Powder Pack straight from the foil to the water sample.
6. Close the vial tightly with the cap and swirl several times to mix the contents.
7. Place the vial in the sample chamber making sure that the marks are aligned.
8. Press TEST key.

Wait for a reaction period of 5 minutes. After the reaction period is finished the measurement starts automatically. The result is shown in the display in mg/l Sulfate.

Appendix A8: Determination of SO_4^{-2}

Procedure:

Insert the adapter for 16 mm Ø vials.

1. Open one white capped vial, add 1 ml of the water sample and close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber.
5. Add the contents of one Vario Nitrate Chromotropic Powder Pack straight from the foil into the same water sample.
6. Close the vial tightly with the cap and invert gently several times (10 x) to mix the contents
7. Place the vial in the sample chamber making sure that the marks are l aligned.
8. Press TEST key. Wait for a reaction period of 5 minutes.

After the reaction period is finished the measurement starts automatically. The result is shown in the display in mg/l Nitrate.

Appendix A9: Determination of PO_4^{3-}

Insert the adapter for 16 mm Ø vials.

1. Open the white cap of one digestion tube PO4-P Acid reagent and add 5 ml of the water sample.

2. Add the contents of one Vario Potassium Persulfate F1 Powder Pack straight from the foil to the vial .

3. Close the vial tightly with the cap and invert several times to mix the contents.

4. Heat the vials for 30 minutes in the preheated reactor at a temperature of 100°C.

5. After 30 minutes remove the vial from the reactor. Allow the vials to cool to room temperature.

6. Open the cooled digestion vial and add 2 ml 1.54 N Sodium Hydroxide Solution to the vial.

7. Close the vial with the cap and invert gently several times to mix the contents.

8. Place the vial in the sample chamber making sure that the l marks are aligned.

9. Press ZERO key.

10. Remove the vial from the sample chamber.

11. Add the contents of one VARIO Phosphate Rgt. F10 Powder Pack straight from the foil to the vial (Note 2).

12. Close the vial tightly with the cap and swirl several times to mix the contents (approx. 10-15 times).

13. Place the vial in the sample chamber making sure that the l marks are aligned.

14. Press TEST key. Wait for a reaction period of 2 minutes.

After the reaction period is finished the measurement starts automatically. The result is shown in the display in mg/l total Phosphate.

Appendix A10: Determination of F^-

1. 0.05 – 2 mg/l F

1. Fill a clean vial (24 mm Ø) with exactly 10 ml of water sample, close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber.
5. Add exactly 2 ml SPADNS reagent solution to the water sample.
2. Caution: Vial is filled up to the top
6. Close the vial tightly with the cap and swirl several times to mix the contents.
7. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press TEST key. The result is shown in the display in mg/l Fluoride.

Appendix A11: Determination of K^+

0.7 – 12 mg/l K

1. Fill a clean vial (24 mm Ø) with 10 ml of the water sample, close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber.
5. Add one Potassium T tablet straight from the foil to the water sample and crush the tablet using a clean stirring rod.
6. Close the vial tightly with the cap and swirl several times until the tablet is dissolved.
7. Place the vial in the sample chamber making sure that the marks are aligned.
8. Press TEST key.

The result is shown in the display in mg/l Potassium.

Appendix A12: Determination of Fe

1. Fill a clean vial (24 mm Ø) with 10 ml of the water sample, close tightly with the cap.
2. Place the vial in the sample chamber making sure that the marks are aligned.
3. Press ZERO key.
4. Remove the vial from the sample chamber.
5. Add one IRON LR tablet straight from the foil to the water sample and crush the tablet using a clean stirring rod.
6. Close the vial tightly with the cap and swirl several times until the tablet is dissolved.
7. Place the vial in the sample chamber making sure that the marks are aligned.
8. Press TEST key.

Wait for a reaction period of 5 minutes. After the reaction period is finished the measurement starts automatically. The result is shown in the display in mg/l Iron (Fe2+/3+).

Appendix A13: Determination of Zn^{2+}

0.02 – 0.9 mg/l Zn

1. Fill a clean vial (24 mm Ø) with 10 ml of the water sample.
2. Add one COPPER / ZINC LR tablet straight from the foil to the water sample, crush the tablet using a clean stirring rod.
3. Close the vial tightly with the cap and swirl several times until the tablet is dissolved.
4. Place the vial in the sample chamber making sure that the marks are aligned.
    1. Press ZERO key. Wait for a reaction period of 5 minutes. After the reaction period is finished the measurement starts automatically.
5. Remove the vial from the sample chamber.

6. Add one EDTA tablet straight from the foil to the prepared vial and crush the tablet using a clean stirring rod.
7. Close the vial tightly with the cap and swirl several times until the tablet is dissolved.
8. Place the vial in the sample chamber making sure that the marks are aligned.
9. Press TEST key. The result is shown in the display in mg/l Zinc.